

SPECIES INTERACTIONS AFFECT THE DISTRIBUTION AND EVOLUTION OF  
MULTIPLE FLORAL TRAITS IN CALIFORNIA NATIVE WILDFLOWERS

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Darwin famously identified that species interactions in a “tangled bank” could influence how species evolve. Yet community ecology – the study of species interactions – and evolutionary biology – the study of phenotypic evolution – have been primarily studied in isolation; as a result, we still have limited knowledge of how ecological interactions cause phenotypic change in multiple types of traits, and how the evolutionary effects of these interactions may vary across communities. In flowering plant communities, co-occurring plant species often share pollinators, which can lead to indirect beneficial facilitation or detrimental competition between plant species for pollination. I hypothesized that interactions between co-occurring plant species might affect the composition of and patterns of selection in flowering plant communities. In this dissertation, I conducted field and greenhouse common garden studies using species in the genus *Clarkia*, which are California-native annual plants that commonly co-occur in multi-species communities and are primarily pollinated by solitary bees that specialize on the genus. In the first chapter, I assessed how interactions among plants that share pollinators might affect communities ecologically, by determining what species can co-occur, and evolutionarily, by affecting ongoing, *in situ* evolution. I conducted a field survey of species co-occurrence patterns and a greenhouse common garden study of trait variation. Two plant species co-occur more frequently than expected by chance alone, and these species have converged in flowering time and diverged

in flower size where they co-occur. In the second chapter, I tested if differences in flowering times observed in *Clarkia* communities minimize competition for pollination by conducting a phenology manipulation experiment with potted plants. Contrary to the general expectation, my results indicated that staggered flowering in these communities does not minimize competition for pollinators and may result from selection from herbivory or water availability. In the third chapter, I conducted the first test for character displacement—the evolution of trait differences where species co-occur relative to where they occur alone—in a trait that mediates many plant-pollinator interactions, floral scent. I developed high-throughput methods for measuring the floral scent of two species of *Clarkia* to examine whether the emission rates of floral volatiles varied across communities that contain one, two, or four *Clarkia* species. I found a pattern consistent with character displacement in the emission rates of eight species-specific compounds, and novel evidence that this evolutionary process can be context-dependent and may occur via multiple pathways in plants. In the fourth chapter, I examined whether interactions between co-occurring plant species in natural communities alter patterns of net- and pollinator-mediated phenotypic selection on floral traits. Across two years, I estimated phenotypic selection on four species and experimentally tested for pollinator-mediated selection on two species in >20 communities that differ in species richness and floral density. Intraspecific competition at high densities and interspecific competition at low densities may affect the evolution of floral traits in these communities. Together, my results indicate that patterns of trait evolution in more species-rich communities are not predictable from simpler communities, and that species interactions can affect the evolution of multiple aspects of a species' phenotype. Because plant-plant interactions could be modifying selection exerted by the abiotic or biotic environment, my results illustrate that species interactions can have wide-ranging effects on species' evolutionary trajectories.

## BIOGRAPHICAL SKETCH

Katherine Elizabeth (Kate) Eisen grew up in Wellesley, Massachusetts. She attended Amherst College and graduated in May 2012 magna cum laude with Distinction with a Bachelor of Arts in Environmental Studies. In Fall 2014, Kate joined Dr. Monica Geber's research group at Cornell University. She defended her Ph.D. in Spring 2020 and will go on to a post-doctoral position at Lund University with Drs. Magne Friberg and Anna Runemark starting in Fall 2020.

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## CHAPTER 1

# ECOLOGICAL SORTING AND CHARACTER DISPLACEMENT CONTRIBUTE TO THE STRUCTURE OF *CLARKIA* COMMUNITIES<sup>1</sup>

### **Abstract**

Despite long-standing interest in the evolutionary ecology of plants that share pollinators, few studies have explored how these interactions may affect communities during both community assembly (ecological sorting) and through ongoing, in situ evolution (character displacement), and how the effects of these interactions may change with community context. To determine if communities display patterns consistent with ecological sorting, we assessed the frequency of co-occurrence of four species of *Clarkia* in the southern Sierra foothills (Kern County, CA, USA). To investigate potential character displacement, we measured pollination-related traits on plants grown in a greenhouse common garden from seed collected in communities with one, two, or four *Clarkia* species. Among the four species of *Clarkia* in this region, the two species that are often found in multi-species communities also co-occur with one another more frequently than expected under a null model. This pattern is consistent with ecological sorting, although further investigation is needed to determine the role of pollinators in shaping community assembly. Patterns of trait variation in a common garden suggest that these two species have diverged in floral traits and converged in flowering time where they co-occur, which is consistent with character displacement. Trait variation across community types also suggests that the process and outcome of character displacement may vary with community context. Because community context appears to affect both the direction and magnitude of

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<sup>1</sup> Eisen, K.E., and Geber, M.A. 2018. Ecological sorting and character displacement contribute to the structure of *Clarkia* communities. *Journal of Evolutionary Biology* 31: 1440–1458.

character displacement, change in more species-rich communities may not be predictable from patterns of change in simpler communities.

## Introduction

Despite the accumulation of evidence of rapid evolution on ecological time scales (e.g. Hairston *et al.*, 2005) and new areas of research that aim to connect community ecology and evolutionary biology (e.g. Rowntree *et al.*, 2011; Crutsinger, 2016; Vellend, 2016; Weber *et al.*, 2017), the ecological and evolutionary processes that structure communities are often studied in isolation (Armbruster, 1995; Johnson & Stinchcombe, 2007). Yet these processes can have the same drivers; how species interact with predators, mutualists, and facilitators can affect both the composition of communities (ecological sorting; e.g. Keddy, 2001; Brooker *et al.*, 2007; Kraft *et al.*, 2015) and the evolutionary trajectories of the species in these communities (character displacement; e.g. Thompson, 2005; Johnson & Stinchcombe, 2007; Laine, 2009). These processes may alter trait distributions within and across communities. Under ecological sorting, traits can be either under-dispersed (habitat filtering, competition, or facilitation) or over-dispersed (competition) in a community (Weiher & Keddy, 1995; Briscoe Runquist *et al.*, 2016), while character displacement generates trait differences in sympatric communities relative to allopatric communities (Brown & Wilson, 1956; Germain *et al.*, 2017). While species interactions play a critical role in structuring communities in the eco-evolutionary dynamics and community genetics frameworks, only a small number of studies have explored how species interactions may contribute to both community assembly and the ongoing evolution of species' traits within these communities (e.g. Armbruster, 1986; Kooyers *et al.*, 2017).

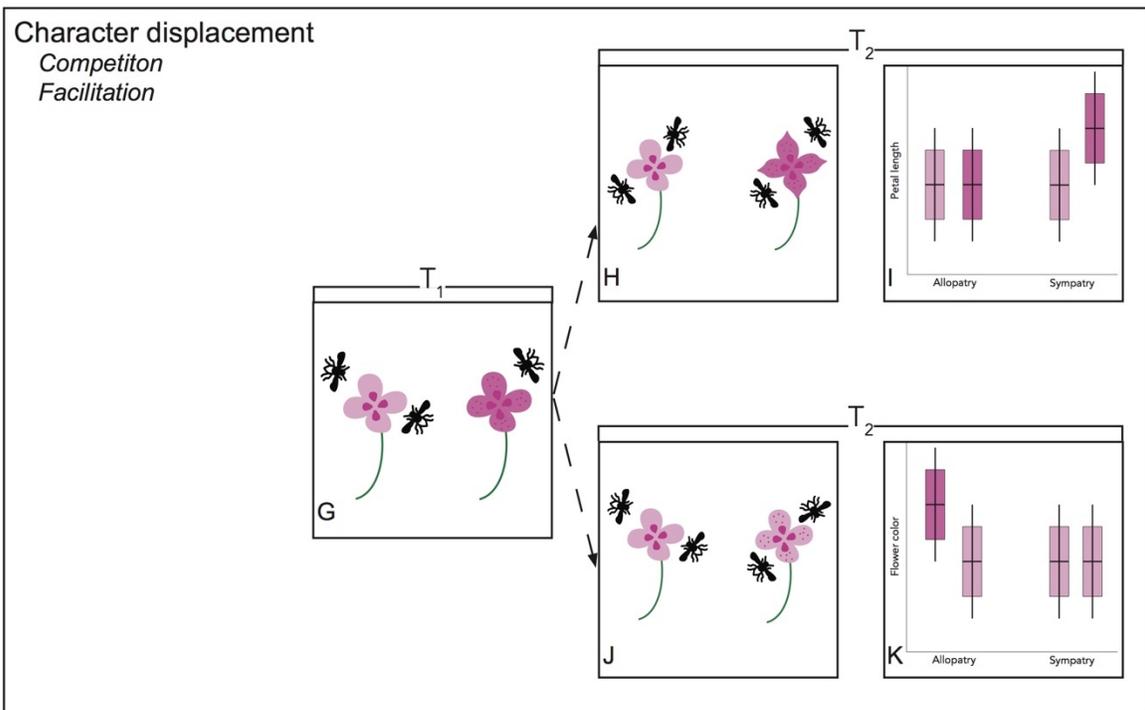
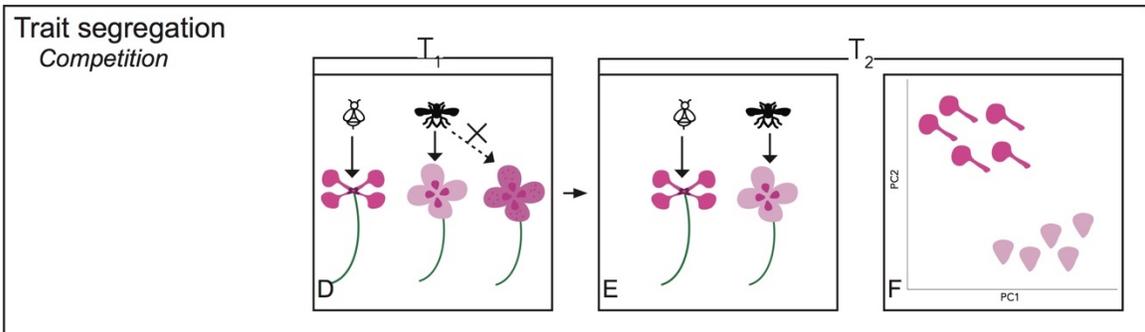
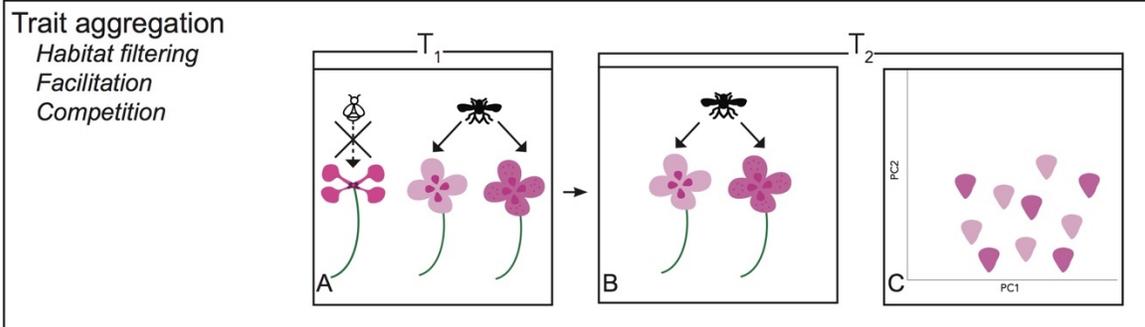
Because most species interact with a broad range of other species, studying pairwise species interactions in isolation does not provide a complete picture of the dynamics of a community (Strauss *et al.*, 2005; Walsh, 2013; terHorst *et al.*, 2015; Mayfield & Stouffer, 2017). Indirect ecological effects, which occur when a third species alters the direct interaction between

two other species (Strauss, 1991; Wootton, 1994), can change the direction and strength of selection on a trait and generate non-additive selection (reviewed in Walsh, 2013; terHorst *et al.*, 2015). As a result, the ecological and evolutionary effects of species interactions depend on the complexity of the community (reviewed in Chamberlain *et al.*, 2014), and the consequences of these interactions vary with the species composition and richness of a community in a number of systems (Caruso, 2000; Rudgers & Strauss, 2004; Arceo-Gómez & Ashman, 2011; Lazaro *et al.*, 2013, 2015).

Communities where multiple plant species share pollinators provide complex yet tractable systems for assessing how species interactions may lead to ecological sorting and character displacement (reviewed in Sargent & Ackerly, 2008; Pellissier *et al.*, 2012). Although plant-pollinator interactions have been primarily studied in terms of direct, pairwise interactions between one plant and one pollinator species (Sargent & Ackerly, 2008; Mitchell *et al.*, 2009), pollinators are often shared among multiple species within a community (Glover, 2007) and are critical for reproduction in most angiosperms (Ollerton *et al.*, 2011). In addition to abiotic and other biotic drivers of community dynamics, pollinator sharing can generate predictable patterns of variation in traits that affect pollination across and within plant communities (Table 1.1; Sargent & Ackerly, 2008; Pellissier *et al.*, 2012). The presence or preferences of pollinators within a community can result in trait similarity (caused by habitat filtering, facilitation, or competition, Figure 1.1 A-C) or trait dissimilarity (caused by competition; Figure 1.1 D-F); shifts in contemporary pollinator-mediated selection where species co-occur relative to where they occur alone can result in *in situ* evolutionary trait change (character displacement; Figure 1.1 G-K).

Patterns of trait variation consistent with pollinator-mediated ecological sorting and

**Figure 1.1. Schematic representation of the effects of ecological sorting and character displacement among plants that share pollinators. Interactions are depicted at an initial time point (T<sub>1</sub>; left column), and the resulting community composition and trait values are depicted at a subsequent time (T<sub>2</sub>; centre and right columns). When a plant species relies upon a pollinator that is not present in an environment, or when a pollinator does not visit a particular plant species (A), the plant will not become established in the community (B); the resulting community members will have similar trait values and clump together in PC space (C). This pattern of trait aggregation can be driven by habitat filtering, facilitation, or competition (Sargent & Ackerly, 2008; Pellissier *et al.*, 2012). When a pollinator's preference for traits associated with one species creates strong competition for pollination (D), the inferior species is excluded from the community (competitive exclusion; E); the resulting community members have different trait values and segregate in PC space (F). Change in species phenotypes in sympatry (character displacement; I and K) can be divergent (G– I) if trait differences reduce competition or increase facilitation [for instance, a floral shape change in the dark pink species on the right from more rounded petals (G) to more pointed petals (H)]. Alternatively, changes in species phenotypes in sympatry can be convergent (G, J, and K) if there is one phenotype associated with competitive dominance [for instance, pollinator preference for the light pink coloured-species causes the dark pink species (G) to evolve to become light pink (J)].**



**Table 1.1.** Evidence for pollinator-mediated ecological sorting and character displacement. Not all studies that provide evidence for character displacement tested all six of the criteria for character displacement articulated by Schluter & McPhail (1992).

<b>Process</b>	<b>Trait(s) of interest</b>	<b>Study system</b>	<b>Reference</b>
Ecological sorting—Trait aggregation	Flower size and shape, flower colour, nectar secretion; arrangement of flowers on inflorescences	9 sp. in White Mountains, Arizona, USA	Brown & Kodric-Brown, 1979
	Floral morphology	771 sp. in western Swiss Alps	Pellissier <i>et al.</i> , 2010
	Flower size and shape, flower colour, stigma-anther separation	<i>Limnanthes</i> sp., <i>Mimulus</i> sp., <i>Clarkia</i> sp. in California, USA	Briscoe Runquist <i>et al.</i> , 2016
	Floral UV pigmentation	35 sister pairs in the Potentilleae tribe (Rosaceae) in North America	Koski & Ashman, 2016
Ecological sorting—Trait segregation	Pollination syndromes	<i>Erica</i> sp. in South Africa	Heystek & Pauw, 2014
	Flower size and shape, flower colour, stigma-anther separation	<i>Limnanthes</i> sp., <i>Mimulus</i> sp., <i>Clarkia</i> sp. in California, USA	Briscoe Runquist <i>et al.</i> , 2016
	Flower size, stigma length, flowering synchrony	21-44 sp. in lowland and montane rain forests in southeastern Brazil	Wolowski <i>et al.</i> , 2016
	Flower colour, morphology, and nectar secretion; flowering time	<i>Costus</i> sp. on Barro Colorado Island, Panama	Schemske, 1981
Character displacement	Size of resin glands and location of reproductive parts	<i>Dalechampia</i> sp. in Central and South America	Armbruster, 1985, 1986
	Flower colour	<i>Phlox</i> sp. in Texas, USA	Levin, 1985; Hopkins & Rausher, 2011, 2012
	Position of staminate and pistillate tissues	<i>Stylidium</i> sp. in western Australia	Armbruster <i>et al.</i> , 1994
	Reduced floral morphology to facilitate selfing	<i>Arenaria</i> sp. in South Carolina and Georgia, USA	Fishman & Wyatt, 1999

(Table 1.1 continues)

Table 1.1 (continued)

<b>Process</b>	<b>Trait(s) of interest</b>	<b>Study system</b>	<b>Reference</b>
Character displacement	Exsertion of reproductive parts	<i>Burmeistera</i> sp. in cloud forests in Ecuador and Costa Rica	Muchhala & Potts, 2007
	Flower colour	<i>Mimulus</i> sp. in California, USA	Grossenbacher & Stanton, 2014
	Flower colour	<i>Leavenworthia</i> sp. in the Central Basin of Tennessee, USA	Norton <i>et al.</i> , 2015
	Flower size	35 sister pairs in the Potentilleae tribe (Rosaceae) in North America	Koski & Ashman, 2016
	Height, flowering time, floral morphology	<i>Mimulus</i> sp. in central Oregon, USA	Kooyers <i>et al.</i> , 2017
	Flower colour	Iochrominae in the Andes	Muchhala <i>et al.</i> , 2014
	Flower size and phenology	<i>Solanum</i> sp. in Mexico	Whalen, 1978

character displacement have been observed in a number of systems (Table 1.1), but there are two key gaps in our understanding of these processes. First, most studies have not collected data both within and across communities in order to determine the relative importance of both processes (but see Koski & Ashman, 2016; Kooyers *et al.*, 2017). Pollinator-mediated ecological sorting may occur more frequently where reproductive interactions among plants are stronger or more specialized (Collins *et al.*, 1983; Gumbert *et al.*, 1999; McEwen & Vamosi, 2010; Pellissier *et al.*, 2010), while pollinator-mediated character displacement may be more common where these interactions are not strong enough to generate filtering or exclusion (Armbruster, 1995; Table 1.1). Second, most plant communities are complex, such that most plant species interact with a number of other plant species via shared pollinators (Olesen & Jordano, 2002; Bascompte *et al.*, 2003). However, previous studies of character displacement have focused on pairwise interactions and have not explored how displacement may change in a more complex community. Because indirect ecological effects can either strengthen or dilute a pattern of selection generated by a pairwise interaction (terHorst *et al.*, 2015), the strength of patterns consistent with character displacement in more complex communities will depend on the nature of the interactions between the additional species present in these communities (Barraclough, 2015; Mayfield & Stouffer, 2017). Studying character displacement in different types of sympatric communities will help to determine whether character displacement generally generates repeatable or non-repeatable patterns of phenotypic change (Germain *et al.*, 2017).

In this study, we assess the potential for ecological sorting and character displacement in communities that contain up to four *Clarkia* species. Species in the genus *Clarkia* often co-occur and share solitary bee pollinators (Lewis, 1953; MacSwain *et al.*, 1973; Singh, 2014); four outcrossing species have overlapping ranges in the southern foothills of the Sierra Nevada (Kern

County, CA). We conducted a greenhouse common garden experiment to measure pollination-related plant and floral traits of progeny of plants from communities that contain one, two or four *Clarkia* species. By eliminating variable environmental effects on trait values, the common garden enabled us to assess whether phenotypic variation has a genetic basis, which is necessary in order to attribute change to character displacement (Losos, 2000), and to compare phenotypes across different, replicated community types. In addition, we conducted road transects to determine the frequencies of species co-occurrence in this region. These data were used to test three, non-mutually exclusive questions about the distribution of pollination-related traits expected if ecological sorting (Q1) and/or character displacement (Q2) occur in these communities, and about how patterns of character displacement might vary across different sympatric communities (Q3):

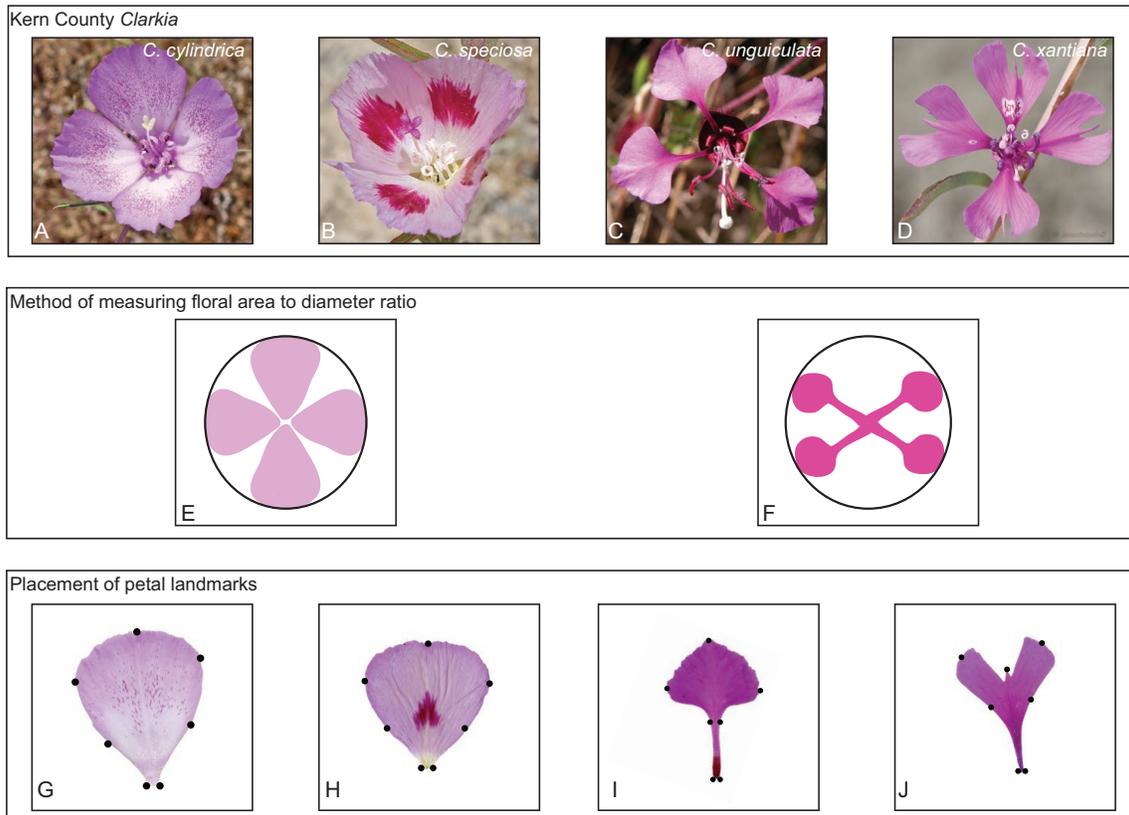
1. Do species' distributions suggest an effect of pollinator sharing during community assembly?
2. Are there genetically-based differences in species' phenotypes in sympatry relative to allopatry that suggest pollinator sharing has generated phenotypic differentiation?
3. Is the magnitude or direction of phenotypic differentiation in sympatry consistent across different multi-species communities?

## **Material and Methods**

*Study system.* Four aspects of communities of *Clarkia* species (Onagraceae) provide an ideal system for investigating the potential for ecological sorting and character displacement. First, *Clarkia* species are more commonly found co-occurring with congeners than occurring alone. While none of the approximately 40 endemic winter annual plant species in the genus have identical ranges in the Western US (Lewis, 1953; Geber & Moeller, 2006), multiple species often

occur in sympatry; a community may contain as many as six *Clarkia* species (Lewis, 1953; MacSwain *et al.*, 1973). Second, *Clarkia* communities persist in the same location for long periods—30-40 years or more (Lewis, 1953; author pers. obs.), creating an opportunity for species interactions to affect the selective environment (Thompson, 2005; Beans, 2014). Third, *Clarkia* are primarily pollinated by solitary bees that specialize on the genus (MacSwain *et al.*, 1973; Moeller, 2005). Co-occurring species receive visits from distinct yet overlapping subsets of these specialist pollinators (MacSwain *et al.*, 1973; Singh, 2014); this degree of resource overlap suggests that while pollinators represent a shared resource, there is an ecological opportunity for specialization (i.e. available niche space) (Pfennig & Pfennig, 2009). Fourth, *Clarkia* species vary in a number of pollination-related traits including floral orientation and flowering time. The ancestral floral orientation in the genus is a bowl, but an open, forward-facing flower has evolved independently multiple times (Sytsma *et al.*, 1990). Co-occurring species also vary in flowering time (Lewis, 1961; Moeller, 2004), which leads to more direct interactions among co-flowering species and indirect interactions among non-co-flowering species that share pollinators across the flowering periods (Waser & Real, 1979). Additionally, two species in northern California display displacement in flower colour where they co-occur (Lewis & Lewis, 1955). Taken together, these four aspects of sympatric *Clarkia* communities suggest that both ecological sorting and character displacement may act on differences in pollination phenotypes and mediate species coexistence.

In the Southern Sierra Nevada (Kern River Canyon, Kern County, CA), communities contain up to four outcrossing *Clarkia* species [*C. cylindrica* ssp. *clavicaarpa* W. Davis, *C. speciosa* ssp. *polyantha* Harlan Lewis and M. Lewis, *C. unguiculata* Lindley, and *C. xantiana* ssp. *xantiana* A. Gray (referred to here as *C. xantiana*); Figure 1.2 A-D]. The species provide



**Figure 1.2.** The four species of *Clarkia* found in Kern County, CA: *C. cylindrica* ssp. *clavica* W. Davis (A), *C. speciosa* ssp. *polyantha* Harlan Lewis and M. Lewis (B), *C. unguiculata* Lindley (C), and *C. xantiana* ssp. *xantiana* A. Gray (D). Schematic representation of the ratio of petal area to floral area in bowl-shaped (E) and open-faced (F) flowers, and the locations on each species of the seven landmarks that were used in the geometric morphometric analysis (G - J). Photos were used with permission from Neal Kramer (A), Aaron Schusteff (B & D), and Harmut Wisch (C).

pollen and nectar rewards for bee visitors, and are not known to hybridize in field populations (MacSwain *et al.*, 1973). Personal observations of inconstant foraging and previous work on tolerance to heterospecific pollen transfer suggest that pollinator sharing may have a cost. Two species vary in their tolerance of heterospecific pollen receipt, and *C. xantiana* plants from multi-species populations were more tolerant of heterospecific pollen than *C. xantiana* plants without a history of exposure to heterospecific pollen (Arceo-Gómez *et al.*, 2016). However, pollen limitation of *C. xantiana* was lower in populations that contained multiple *Clarkia* species relative to populations where *C. xantiana* occurs alone, indicating that pollinator sharing may be facilitative (Moeller, 2004). These species are all primarily outcrossing; the flowers of all four species are protandrous and herkogamous, with eight anthers maturing over 2-3 days before the stigma becomes receptive (Lewis, 1953). Outcrossing rates in *C. unguiculata* range from 0.79 to 1.0 (Vasek, 1965; Ivey *et al.*, 2016), and outcrossing rates range from 0.59 to 0.85 in *C. xantiana* (Moeller *et al.*, 2012; Ivey *et al.*, 2016). The species vary in flowering period and floral orientation: *C. cylindrica* (bowl-shaped flower) and *C. unguiculata* (open-faced flower) flower in early-mid May (Figure 1.2 A & 1.2 C), while *C. speciosa* (bowl-shaped flower) and *C. xantiana* (open-faced flower) flower in early-mid June (Figure 1.2 B & 1.2 D; Moeller, 2004; Singh, 2014).

Previous work has confirmed that *Clarkia* in the Kern River Canyon share pollinators (MacSwain *et al.*, 1973; Moeller, 2006; Singh, 2014). In surveys of pollinator visitation to communities that contained three or four *Clarkia* species conducted in 2010 and 2011, Singh (2014) found that each of the four *Clarkia* species received significant pollination services (defined as five percent or more of all visits) from two to six (mean: 4) bee genera. In 2010, five species of bees (*Apis mellifera*, *Halictus farinosus*, *Hesperapis regularis*, *Lassioglossum*

*dialictus*, *Lassioglossum pullilabre*) were significant pollinators of two or more species of *Clarkia*, and in 2011, three species of bees (*A. mellifera*, *H. farinosus*, *H. regularis*) were significant pollinators of two or more species of *Clarkia*. While these visitors are likely to differ in their pollination effectiveness and efficiency, previous analysis of pollen loads of visitors to *C. xantiana* indicated that four *Clarkia* specialist species, including *Lassioglossum pullilabre* and *Hesperapis regularis*, had the highest potential to be significant pollinators of *C. xantiana* (Moeller, 2005). These results indicate that there is considerable overlap in the visitors that are likely to contribute significantly to the pollination of these plant species; this overlap may drive ecological sorting and character displacement.

*Study overview.* This study addresses two non-mutually exclusive questions about the potential for ecological sorting and character displacement in *Clarkia* communities. To establish patterns of species' co-occurrence and determine how they relate to species' traits, we conducted road transects through the range of *C. xantiana*. Using data points from the areas where all four species occur, we determined the frequency of different types of communities, and the frequencies with which each species occurred in each type of community. To determine if species were commonly found with other *Clarkia* or with particular species of *Clarkia*, we calculated the frequencies with which each pair of species co-occurred. To determine if species' phenotypes exhibit genetically-based differences in sympatry relative to allopatry (character displacement), we conducted a greenhouse common garden experiment with open-pollinated maternal seed families collected from communities that contain one, two, or four *Clarkia* species. Trait data from these plants were used to test for heritable variation and for trait differences across community types.

*Species co-occurrence patterns: survey methods.* To determine the frequencies with which

species co-occur, we conducted transects along paved and dirt roads throughout the range of *C. xantiana* that were accessible by car in late April and early May of 2017. We restricted our transects to the range of *C. xantiana* because it has the smallest range out of the four species commonly found in Kern County (Jepson, 1993) and because its range has been well-documented by previous studies (Eckhart & Geber, 1999). These transects covered approximately 140 miles of roads in Kern and Tulare counties in California. We stopped every 0.5 miles ( $N = 316$  total stops) and recorded the *Clarkia* species present within 25 meters of the location of the vehicle along both sides of the road. Roadside communities were used in this study because the species present in every roadside population we have studied for more than twenty years in this region (author, pers. obs.) have also been present on the steep slopes above the roadcut in less disturbed habitats, and the species present along the road have not varied from year to year. The GPS coordinates of each stop were recorded using Google Maps. In addition to the four species of *Clarkia* commonly observed in this region, we observed *C. dudleyana* (one stop), *C. rhomboidea* (one stop), *C. xantiana* ssp. *parviflora* (one stop), and *C. exilis* (eight stops). Because these species were observed at low frequencies and because *C. xantiana* ssp. *parviflora* and *C. exilis* are predominantly self-pollinating (Vasek, 1958; Moore & Lewis, 1965), these occurrences were excluded from our dataset. For the purposes of data analysis, we chose to restrict our dataset to the subset of stops made in the Kern River Canyon and Oiler Canyon ( $N = 143$  total stops), as these areas are bounded by significant topological features where all four outcrossing species of *Clarkia* commonly observed in the region are known to occur.

*Species co-occurrence patterns: data analysis.* We used the species co-occurrence data to conduct three sets of analyses. First, motivated by the observation that multiple, closely related *Clarkia* species often co-occur (Lewis, 1953; MacSwain *et al.*, 1973), we calculated the

proportion of the communities that contained one, two, three, or four species.

Second, to determine whether each species is more commonly found alone or with other species, we calculated the number of communities where each species occurred alone, and the number of communities where the species co-occurred with one or more other species. Because we found that 50% of communities with *Clarkia* contained one species and the remaining 50% of communities with *Clarkia* contained two or more species (see Results), our null expectation was a 50-50 distribution of single- and multi-species communities; if co-occurrence is neither favoured nor disfavoured for a given focal species, then we would expect that the distribution of single- and multi-species communities for that species will follow the overall 50-50 pattern observed for all *Clarkia* in this region. This null expectation should be insensitive to the relative rarity of a species—a rare species should be rare at both community types, assuming there was no detection bias against communities that contained only the rare species. To test this null hypothesis, we conducted exact tests on the frequencies of these two community types for each species using the `xmulti` function in the `XNomial` R package (Engels, 2015). This function performs a multinomial Goodness-of-Fit Test by comparing observed values to expected values and returns the log-likelihood ratio and *P* value. A non-significant test result indicates that a species occurs alone and with other species with equal frequencies, while a significant test result indicates that the species is more commonly observed alone or with other species.

Third, to determine if certain combinations of species are found together more frequently than others, we calculated C-scores (Checkerboard scores; Stone & Roberts, 1990) using the `EcoSimR` R package (Gotelli *et al.*, 2015). The C-score for a pair of species is calculated as:

$$C_{AB} = (R_A - SS)(R_B - SS) \quad (1)$$

$R_A$  and  $R_B$  are the numbers of communities that contain species A or B, respectively, and  $SS$  is

the number of communities that contain both species. The minimum possible C-score of 0 occurs when species are maximally aggregated, while the maximum possible C-score of  $R_A R_B$  occurs when SS is 0 and species are maximally segregated; higher C-scores therefore indicate less co-occurrence between species. We tested whether each pair of *Clarkia* species was segregated or aggregated using the `cooc_null_model` function with the algorithm `sim2`, which allows for fixed row and equiprobable columns within the co-occurrence matrix (Veech, 2014). For each pairwise comparison, we present both C-scores, where the magnitude of the score depends on sample sizes, and the Standardized Effect Size, which allows for meaningful comparisons of association strengths across species pairs.

*Common garden experiment: source community selection.* Because the purpose of our common garden was to determine if species' phenotypes exhibit genetically-based differences in sympatry relative to allopatry, we selected two replicates of each of seven unique types of source communities for a total of 14 communities (each containing one, two, or four species of *Clarkia*; Table A.1). The two-species communities contained either *C. cylindrica* and *C. unguiculata* (both early flowering species) or *C. speciosa* and *C. xantiana* (both late flowering species). The 14 communities are distributed throughout the Kern River Canyon; the species compositions of these communities are not a function of community elevation and/or easting (the eastward-measured distance in a Universal Transverse Mercator zone) (ANOVAs; elevation:  $F_{6,7} = 0.447$ ,  $P = 0.827$ ; easting:  $F_{6,7} = 2.103$ ,  $P = 0.177$ ). Seeds from all species and communities were collected in 2014, except seeds from one community containing *C. speciosa* and *C. xantiana* (Site 8) were collected in 2010. One to several fruits per plant were collected from 10 haphazardly chosen plants of each species at each community, as any individual plant may produce one to over 100 fruits.

*Common garden experiment: germination and growth.* We germinated seeds of the 10 open-pollinated maternal families collected per species per community. To break dormancy, on November 24, 2014, 50 seeds per family were placed on moist filter paper in a petri dish, wrapped in parafilm, and stratified at 5°C for seven days and then held at 23°C for three to five days prior to planting. On December 1-5, 2014, we transplanted germinants into 656 ml<sup>3</sup> Cone-tainers (Stuewe & Sons, Tangent, Oregon, USA) filled with a 1:1 mix of fritted clay (Oil Dri, Softco Mead, Elmira, New York, USA) and Metro-Mix soil (Sun Gro Horticulture, Agawam, Massachusetts, USA) (Runions and Geber 2000). Each pot initially contained two germinants (from the same family) and pots were thinned after four weeks to contain one plant. The experiment contained 30 plants per species per community (three plants/family from each of 10 families), for a total of 720 plants. The pots' positions on the greenhouse bench were randomized. Each plant was watered every other day and fertilized with 72 g/L 21-5-20 formula. Plants were exposed to supplemental light (16 h days), and maintained at 23-25°C during the day and 19-21°C at night.

*Common garden experiment: trait measurement.* We recorded two plant-level traits that affect pollination phenotypes: date of first flower and height. Pollinators often generate selection on flowering phenology (Elzinga *et al.*, 2007), a trait that affects pollinator visitation and mate availability (Kudo, 2006). While greenhouse growing conditions may alter plant development schedules, variation in flowering phenology in a greenhouse is correlated with observed variation in flowering time in field populations of *C. xantiana* (Gould *et al.*, 2014). Although plant height is a vegetative trait that may evolve in tangent with or independently from floral traits (Conner *et al.*, 2014), it can contribute to pollinator attraction: tall plants receive more visits from bird (Dudash *et al.*, 2011) and insect (Lortie & Aarssen, 1999) pollinators.

We measured five floral traits that affect pollinator attraction and pollination efficiency: style length, floral diameter, petal area, the ratio of petal area to floral diameter, and petal shape. Style length affects pollination efficiency and the degree of outcrossing by determining where pollen is placed on pollinators and how pollinators make contact with a flower's reproductive organs (Armbruster *et al.*, 1994; Mazer *et al.*, 2016). Flower size (Strauss *et al.*, 1996; Parachnowitsch & Kessler, 2010) and shape traits (Gómez *et al.*, 2006) increase pollinator attraction, and can also affect pollination efficiency (Galen & Newport, 1987).

Due to early mortality of some plants, trait measurements were not made on all 30 of the plants grown per species per community. However, measurements were made on 16 or more plants per species per community for 18 of the 24 community x species combinations (Table A.1). We measured two plant and four floral traits *in vivo*. On the day of first flowering, we recorded the date and plant height. We used calipers to measure style length and floral diameter on 1-4 female phase flowers per plant. These traits were consistently measured in female phase because herkogamy causes both style length and petal expansion to differ between male and female phase (KE, personal observation). The petals from these flowers were removed and pressed onto sheets of cardstock and scanned at a resolution of 2,400 pixels/inch using an Epson Perfection V550 Photo Color Scanner (Epson America, Inc., Long Beach, CA USA). To generate another measure of flower size, we used the scanned petal images to measure petal area. We used Adobe Photoshop CS6 Version 13.0.6 (Adobe Systems Incorporated, San Jose, CA USA) to remove the background from each image. We then measured and took the average of the area of 3 or 4 petals per flower using NIH ImageJ (<http://rsb.info.nih.gov/nih-image/>).

While corolla tube length is often measured to assess both flower shape and size, we did not measure corolla length or floral depth because the open-faced flowers (*C. unguiculata* and *C.*

*xantiana*) do not have a tube or bowl component. Instead, we created a ratio to compare the area of the petals to the area at the opening of the flower:

$$\text{Area: diameter ratio} = \frac{4 \times \text{Area}_{\text{petal}}}{\left(\frac{\text{Diameter}_{\text{flower}}}{2}\right)^2 \times \pi} \quad (2)$$

Higher values of this ratio indicate flowers with petal areas that are close to or exceed the area of the circle at the opening of the flower, while lower values indicate flowers with petal areas that are smaller than the area of this circle. When a bowl-type and open-type flower have the same floral diameter, the bowl-type flower will have a greater petal area to floral diameter ratio (Figure 1.2 E) relative to the open-type flower (Figure 1.2 F). This difference reflects the greater depth of the bowl-type flower, which is created by the larger petals.

To measure petal shape independently of petal size, we used geometric morphometric methods (Claude, 2008). Because linear measurements of floral traits (i.e. flower width, corolla tube depth) do not account for the geometric relationships between floral dimensions, they cannot be used to visualize shape differences (Adams & Rohlf, 2000). Geometric morphometric analyses retain the geometric relationships between aspects of shape by generating shape variables from the  $x,y$  coordinates of unique points that can be identified on all specimens (landmark analysis). Any differences in rotation, translation, and scale are held constant, such that any remaining differences are the result of differences in shape (Zelditch *et al.*, 2004).

To perform landmark analysis, we first identified and digitized the location of  $N = 7$  landmarks along the edges of the petal in each species (Figure 1.2 G-J). The amount of measurement error associated with generating the landmarks was assessed by re-digitizing the location of the landmarks for a set of  $N = 20$  petals of each species using NIH ImageJ (<http://rsb.info.nih.gov/nih-image/>). The percentage of error was calculated as the ratio of the within-measurement component of variance to the sum of the within- and among-measurement

variance component, and the variance components were derived from a one-way ANOVA with the individual petal as the factor. Measurement error for each species ranged from  $< 0.01\%$  to  $8.85\%$ . We performed a partial generalized Procrustes superimposition on the landmark data for all samples of all species (pgpa function in Claude, 2008), and we used an orthogonal projection to generate a Euclidean approximation of the coordinates in the tangent shape space (orp function in Claude, 2008). We performed a principal component analysis on the transformed data, and we included the first two PCs as floral trait variables in our analyses because these two axes together accounted for over  $90\%$  of the variation in the data. PC1 accounted for  $58.7\%$  of the variation in the landmark data; lower values along PC1 correspond to a pentagonal teardrop shape while higher values correspond to a club shape. PC2 accounted for  $32.2\%$  of the variation; lower values along PC2 correspond to a diamond shape while higher values correspond to a heart shape (Figure A.1).

*Common garden experiment: statistical analysis.* All models were analysed in R (R core team 2016) using the lme4 package (Bates *et al.*, 2014). Models were assessed to ensure normally distributed residuals with homogenous variance. All models were general linear mixed effects models, with the exception of generalized linear models fit with a Poisson distribution for the trait of the date of first flower, which was expressed as a count since the start of the experiment (see Table A.2 for a list of all models). The significance of fixed effects in linear mixed models was assessed using the anova function in the lmerTest package ver. 2.0-29 (Kuznetsova *et al.*, 2015) to perform type III  $F$  tests using the Kenward-Roger approximation for the denominator degrees of freedom. When ANOVAs returned significant  $F$  values, we used Tukey's honest significant difference tests to which group means were significantly different using the lsmeans function with the pairwise option in the lsmeans package (Lenth, 2016). We assessed the

significance of random effects and Poisson-distributed response variables using a chi-square test (with 1 d.f.) of twice the difference in the log-likelihood of models with the factor of interest versus without the factor of interest [using restricted maximum likelihood estimation (REML)] (Bates *et al.*, 2014).

*Character displacement: Analysis of heritable trait variation.* To determine if trait variation has a heritable component, we analysed the sources of variation for each floral and plant trait (models described in Table A.2). When the response variable was a floral trait, data from 2-4 female-phase flowers per plant were used as replicates in the analysis (*N<sub>C. cylindrica</sub>* = 214 flowers; *N<sub>C. speciosa</sub>* = 200 flowers; *N<sub>C. unguiculata</sub>* = 300 flowers; *N<sub>C. xantiana</sub>* = 154 flowers). For analyses of the plant traits, some traits were either square root or cube root transformed to ensure normally distributed model residuals with homogenous variance (Table A.3; models in Table A.2). Data from individual plants within families were used as replicates in the analysis (*N<sub>C. cylindrica</sub>* = 147 plants; *N<sub>C. speciosa</sub>* = 119 plants; *N<sub>C. unguiculata</sub>* = 168 plants; *N<sub>C. xantiana</sub>* = 105 plants). The significance of plant (floral traits only) and family (floral and whole plant traits) were tested with a chi-square test (described above). A significant plant or family term indicates that there is substantial variation in that trait within that level of the analysis.

*Character displacement: analysis of trait differences within species and across community types.* To test whether species' traits vary where they co-occur with other *Clarkia* species relative to where they occur alone, we compared all pairs of species. The models that contrast *C. cylindrica* with *C. unguiculata* and *C. speciosa* with *C. xantiana* compare their traits at communities with one, two, or four species, while the models that contrast the four other pairs of species compare their traits at one- and four-species communities because we did not grow seeds from two-species communities that contained these combinations of species (see Table A.1).

To first determine if community type affects patterns of trait expression among correlated floral traits, we used MANOVA (models in Table A.2). We analysed floral traits separately from whole plant traits because trait covariation was higher among floral traits than across all traits (results not shown). We applied transformations to some traits to ensure normally distributed model residuals with homogenous variance. For the *C. cylindrica* – *C. unguiculata* contrast, petal area was log transformed. For the *C. cylindrica* – *C. speciosa* contrast, style length was square root transformed.

When there was a significant interaction term in the MANOVA, we ran ANOVAs with individual floral traits as the response to determine which of the traits caused the observed patterns; we also ran ANOVAs with individual plant-level traits as the response to determine if community type affects these traits (models in Table A.2). When the ANOVAs returned significant *F* tests, we computed Tukey's honest significant difference tests (described above) to determine the community types where the species differed in the trait in the model. We then tested the null hypothesis that these differences between species would be equivalent across community types by specifying a matrix of contrasts and used the `ghlt` function in the `multcomp` package for R (Hothorn *et al.*, 2008) to perform Generalized Linear Hypothesis Tests that were corrected for multiple comparisons. These tests compare the difference between a pair of species at a given community type to the difference between those two species at another community type. In other words, these tests determine if the differences between species are consistent or variable across community types. For instance, if species are more phenotypically divergent where they co-occur relative to where they occur alone, the difference between species in sympatry will be significantly larger than the difference between species in allopatry.

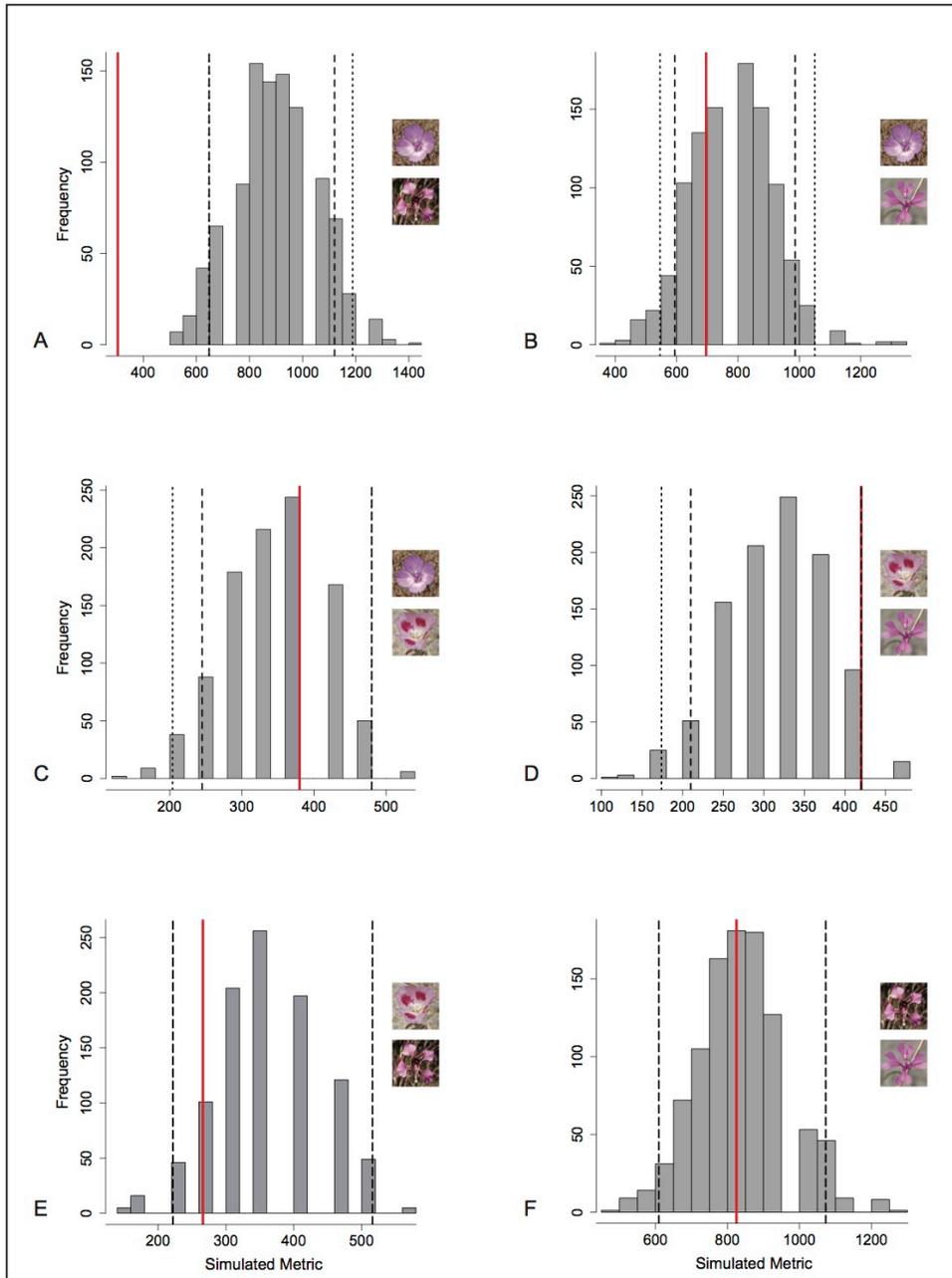
## Results

### *Ecological sorting: species co-occurrence patterns*

Along road transects through the Kern River Canyon and Oiler Canyon, we observed one or more species of *Clarkia* at 84 of 142 stops. Of these 84 stops that contained *Clarkia*, 42 stops (50.0 %) contained one species, 34 stops (40 %) contained two species, and 8 stops (10 %) contained three species. None of our stops contained all four species commonly observed in this area.

To determine if each species is more commonly observed alone or with other species, we performed exact tests on the number of single-species communities vs. the number of multi-species communities for each species. *Clarkia unguiculata* frequently co-occurred with other species (10 alone communities, 34 multi-species communities; log-likelihood ratio = -6.91,  $P = 0.0003$ ), as did *C. cylindrica* (9 alone communities, 32 multi-species communities; log-likelihood ratio = -6.84,  $P = 0.0004$ ). However, *C. speciosa* occurred alone roughly as frequently as it co-occurred with other species (5 alone communities, 8 multi-species communities; log-likelihood ratio = -0.35,  $P = 0.58$ ), and *C. xantiana* was also equally common at single and multi-species communities (18 alone communities, 18 multi-species communities; log-likelihood ratio = 0,  $P = 1$ ).

We calculated the C-scores of all species pairs to determine whether pairs of species' distributions were segregated, aggregated, or random. The observed C-score for *C. unguiculata* and *C. cylindrica* was significantly lower than the lower bound of the 95 % confidence interval around the simulated C-score, (Standardized Effect Size = -3.97;  $P < 0.001$ ), which indicates that these species' distributions are aggregated (Figure 1.3 A). For the other five species pairs, the observed C-score fell within the 95 % confidence interval around the simulated C-score,



**Figure 1.3.** Histograms depicting the distribution of simulated C-score values (grey bars) and the observed value for the original data (red line), with the vertical long-dash lines indicating the 95 % one-tailed cutpoints, and the short-dash lines indicating the 95 % two-tailed cutpoints. *C. cylindrica* and *C. unguiculata* (A) are significantly aggregated (Standardized Effect Size [SES]: -3.97, lower-tail  $P < 0.001$ ), while all other species pairs are not distributed differently from the null expectation; *C. cylindrica* and *C. xantiana* (B; SES: -0.78; lower-tail  $P = 0.272$ ); *C. cylindrica* and *C. speciosa* (C; SES: 0.45; lower-tail  $P = 0.788$ ); *C. speciosa* and *C. xantiana* (D; SES: 1.56; upper-tail  $P = 0.118$ ); *C. speciosa* and *C. unguiculata* (E; SES: -1.26; lower-tail  $P = 0.172$ ); *C. unguiculata* and *C. xantiana* (F; SES: -0.08; lower-tail  $P = 0.563$ ).

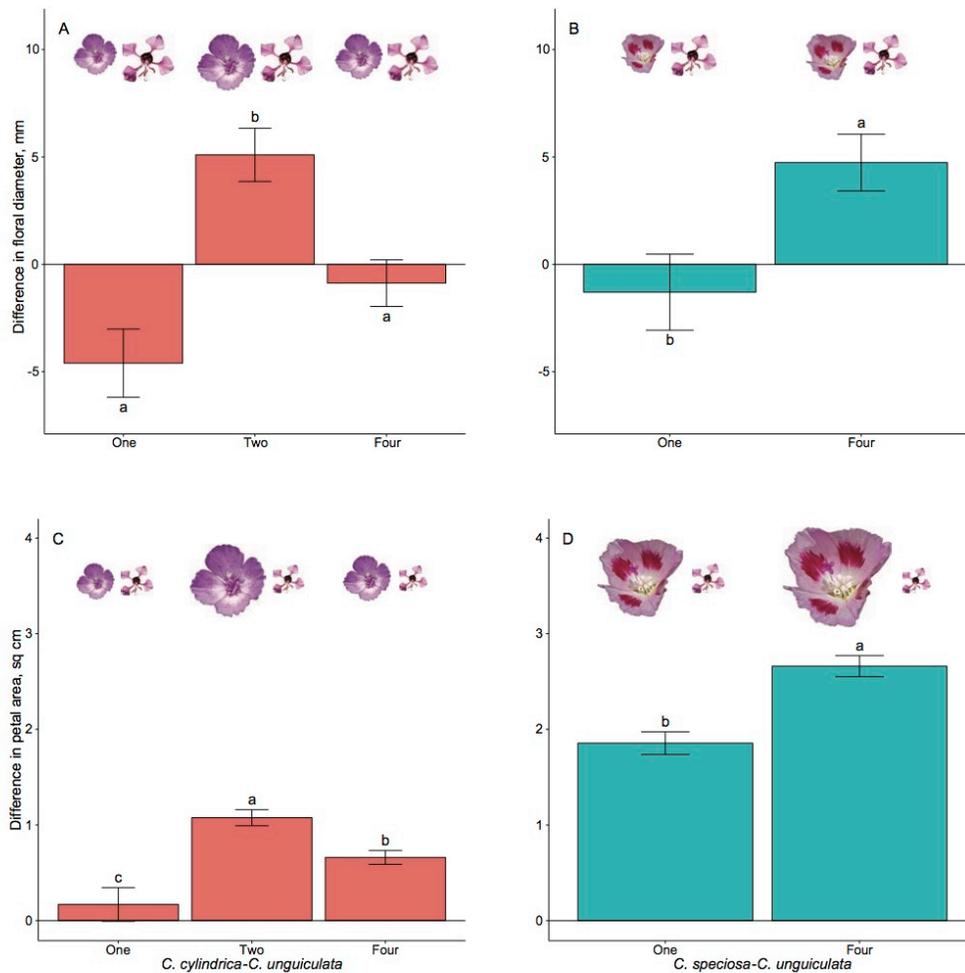
indicating that the distributions of these species pairs are not significantly segregated or aggregated (Figure 1.3 B-F).

*Character displacement: genetic variation in traits.*

Because the evolutionary process of character displacement requires genetic variation in a trait, we assessed whether each pollination-related trait exhibited variation among open-pollinated maternal families in a greenhouse environment. There was significant variation on the family and plant levels for all six floral traits of all four species (Table A.3), which indicates these traits could be targets of character displacement. The plant traits (height and date of first flower), however, did not exhibit significant family-level variation in all of the species (Table A.3). Height varied among families of *C. unguiculata* and *C. cylindrica*, while date of first flower varied among families of *C. unguiculata* and *C. xantiana*.

*Character displacement: floral trait differences within species and across community types.*

MANOVA models of all six floral traits had a significant species x community type term for two pairs of species (Table A.4). ANOVA models of *C. cylindrica* and *C. unguiculata* returned a significant species x community type interaction term for two floral traits: floral diameter ( $F_{2, 20.29} = 12.25$ ;  $P = 0.0003$ ) and petal area ( $F_{2, 12.94} = 12.59$ ;  $P = 0.0009$ ). The floral diameter of *C. unguiculata* was 17 % larger than that of *C. cylindrica* at one-species communities, but *C. cylindrica* had 16 % larger floral diameter than *C. unguiculata* at two-species communities (see images of flowers in Figure 1.4 A); this change in the difference between *C. cylindrica* and *C. unguiculata* resulted from a 35 % increase in the diameter of *C. cylindrica* at two-species communities relative to the diameter at one-species communities [Table A.5; mean  $\pm$  1 SE of traits by community type (Table A.8) and by community (Table A.9)]. The differences between the species at one- and two-species communities are similar in size but opposite in direction



**Figure 1.4.** The average differences  $\pm 1$  SE between species for the two floral traits that show significant differences across community types (number of *Clarkia* species present): floral diameter (A, B), and petal area (C, D). The images of the flowers above each bar are scaled to the precise relative size of the floral trait at that community type. For comparisons of *C. cylindrica* and *C. unguiculata* (red; A, C), differences presented are the value of *C. cylindrica* minus the value of *C. unguiculata*, such that positive values indicate communities where *C. cylindrica* has a larger trait value than *C. unguiculata*. For comparisons of *C. speciosa* and *C. unguiculata* (blue; B, D), differences presented are the value of *C. speciosa* minus the value of *C. unguiculata*, such that positive values indicate communities where *C. speciosa* has a larger trait value than *C. unguiculata*. Differences with different lowercase letters above or below the error bars within a panel indicate that these estimates are significantly different from each other ( $P < 0.05$ ).

(Figure 1.4 A; Table A.5). Both of these differences are over 400 % larger than the difference between the species at four-species communities, which was not different from zero (Figure 1.4A; Table A.5). The difference in the petal area values of the two species was not different from zero at one-species communities, but the petals of *C. cylindrica* were 103 % larger than the petals of *C. unguiculata* at two-species communities and 73 % larger than the petals of *C. unguiculata* at four-species communities (Figure 1.4 C); the difference between the species in petal area at two-species communities was 466 % larger than at one-species communities, while the difference between the species at four-species communities was 233 % larger than at one-species communities (Figure 1.4 C; Table A.5).

The other species pair with a significant species x community type interaction term in the MANOVA model of all floral traits was *C. speciosa* and *C. unguiculata*. ANOVA models of this species pair also returned significant interaction terms for the same two floral traits, floral diameter ( $F_{1,9.24} = 7.82$ ;  $P = 0.020$ ), and petal area ( $F_{1,16.37} = 30.16$ ;  $P < 0.0001$ ). The floral diameters of *C. unguiculata* and *C. speciosa* were not different from each other at one-species communities, but *C. speciosa* had a 16 % larger diameter than *C. unguiculata* at four-species communities (Figure 1.4 B; Table A.6). The difference in floral diameter between the species at four-species communities was 377 % larger than the insignificant difference between them at one-species communities (Figure 1.4 B; Table A.6). The petals of *C. speciosa* were 165 % larger than the petals of *C. unguiculata* at one-species communities and 294 % larger than the petals of *C. unguiculata* at four-species communities (Figure 1.4 D); the difference in petal area between the species at four-species communities was 55 % larger than the difference between the species at one-species communities (Figure 1.4 D; Table A.6).

*Character displacement: plant trait differences within species and across community types.* For

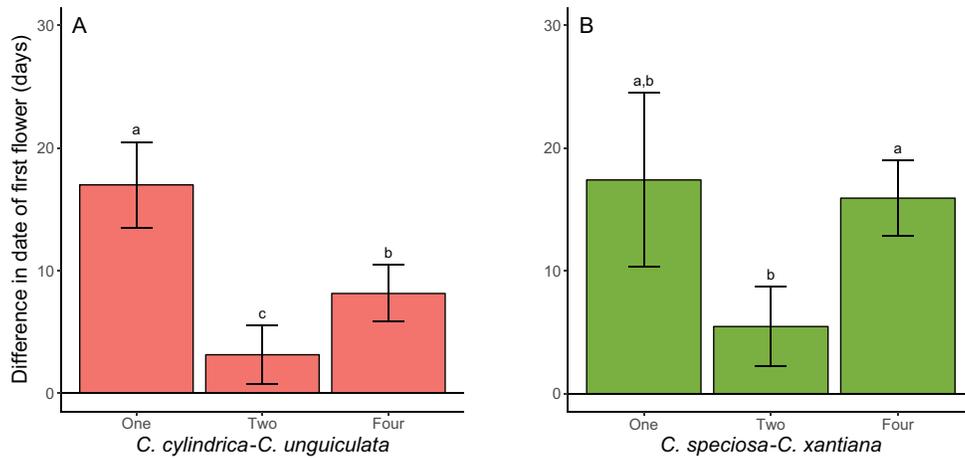
the date of first flower, there was a significant interaction between community type and species for models comparing two pairs of species: *C. cylindrica* and *C. unguiculata* ( $\chi^2 = 20.58$ ;  $P < 0.0001$ ), and *C. speciosa* and *C. xantiana* ( $\chi^2 = 33.59$ ;  $P < 0.0001$ ). *Clarkia cylindrica* flowered later than *C. unguiculata* at all community types (positive differences in date of first flower; Figure 1.5 A), but the differences between the species were not constant across community types. Flowering at two-species communities was 123 % more aggregated relative to flowering at one-species communities, and 153 % more aggregated relative to flowering at four-species communities. Flowering at four-species communities was 16 % more aggregated than at one-species communities (Figure 1.5 A; Table A.7). In the other significant species pair, *C. speciosa* flowered later than *C. xantiana* at all community types (positive differences in date of first flower; Figure 1.5 B), but flowering at two-species communities was 204 % more aggregated relative to four-species communities (Figure 1.5 B; Table A.7).

The interaction between community type and species on plant height was not significant for any of the pairs of species.

## **Discussion**

### *Patterns of species co-occurrence and trait variation among pollinator-sharing plants*

The first goal of our study was to determine if ecological sorting and character displacement might occur in communities of *Clarkia* species that share pollinators in the southern foothills of the Sierra Nevada. We found patterns of species co-occurrence that are consistent with ecological sorting as well as trait variation that is consistent with character displacement, although further studies are needed to definitively attribute these patterns as outcomes of pollinator sharing among co-occurring plants. These results follow from previous studies that have found evidence for ecological sorting and character displacement among plants



**Figure 1.5.** The average differences  $\pm 1$  SE between species in the date of first flowering. For comparisons of *C. cylindrica* and *C. unguiculata* (red; A), differences presented are the value of *C. cylindrica* minus the value of *C. unguiculata*, such that positive values indicate communities where *C. cylindrica* has a larger trait value (later flowering) than *C. unguiculata*. For comparisons of *C. speciosa* and *C. xantiana* (green; B), differences presented are the value of *C. speciosa* minus the value of *C. xantiana*, such that positive values indicate communities where *C. speciosa* has a larger trait value (later flowering) than *C. xantiana*. Differences with different lowercase letters above or below the error bars within a panel indicate that these estimates are significantly different from each other ( $P < 0.05$ ).

that share pollinators (Table 1.1), although most studies have not collected data both within and across communities in order to determine the relative importance of both processes in the same system (e.g. Briscoe Runquist *et al.*, 2016; but see Koski & Ashman, 2016; Kooyers *et al.*, 2017). Here we discuss what types of species interactions might generate the observed patterns in species co-occurrence and trait variation, and what additional data are needed to definitively attribute these patterns to pollinator sharing.

The aggregated distributions of two *Clarkia* species, *C. unguiculata* and *C. cylindrica*, could result from habitat filtering, competition, or facilitation (Briscoe Runquist *et al.*, 2016). If these species are both affiliated with the same partner species (e.g. a pollinator), or if these species have similar abiotic niches, then a biotic or abiotic habitat filter may be responsible for the observed pattern (Weiher & Keddy, 1995; Sargent & Ackerly, 2008). A biotic habitat filter seems unlikely in this system because *Hesperapis regularis*, the key pollinator that comprised upwards of 24 % of visits to *C. unguiculata* and about 60 % of visits to *C. cylindrica* in 2010 and 2011, is also a significant pollinator of *C. xantiana* and *C. speciosa* (Singh, 2014). This suggests that the distribution or foraging preferences of *H. regularis* do not promote the co-occurrence of *C. cylindrica* and *C. unguiculata* more so than the other *Clarkia* species. Because we did not measure aspects of the abiotic environment where these species do and do not co-occur, we cannot rule out the potential for an abiotic filter. Constructing species distribution models for these two species would represent one way of determining whether the aggregation of these species is correlated with variation in the abiotic environment.

If the aggregated pair of species is both more attractive to pollinators than the other *Clarkia* species in the region, then their competitive superiority may lead to their co-occurrence (Mayfield & Levine, 2010; HilleRisLambers *et al.*, 2012; Godoy *et al.*, 2014; Briscoe Runquist

*et al.*, 2016). However, out of the four species that co-occur in this region, *C. cylindrica* received the smallest number of pollinator visits in two years of observations in four-species communities (Singh, 2014); this result suggests that *C. unguiculata* and *C. cylindrica* are not significantly more attractive to pollinators than *C. xantiana* and *C. speciosa*. Experimental studies that assess how different species interact are needed to determine if competition is driving the aggregation of species with similar traits (Godoy *et al.*, 2014; Heystek & Pauw, 2014; Kraft *et al.*, 2015b).

Finally, facilitation may be driving the aggregation of these species if the two species can together attract and or maintain a larger pool of pollinators where they co-occur (Moeller, 2004; Geber & Moeller, 2006; Sargent & Ackerly, 2008; Briscoe Runquist *et al.*, 2016). Because *Clarkia cylindrica* and *C. unguiculata* share a number of key pollinators (Singh, 2014), their overlap in flowering time could contribute to joint pollinator attraction. While aggregation of flowering schedules in some communities reflects phylogenetic constraints (e.g. Smith-Ramirez *et al.*, 1998), co-flowering species have experienced increased pollinator visitation and seed set in a number of communities in response to the greater availability of floral resources (Brown & Kodric-Brown, 1979; Schemske, 1981; Ashton *et al.*, 1988; Gross *et al.*, 2000; Moeller, 2004) or to the availability of rewards (Laverty, 1992; Johnson *et al.*, 2003; Ghazoul, 2006). While trait similarities are expected to promote joint pollinator attraction, trait differences that promote pollinator constancy and decrease heterospecific pollen transfer may also be critical to facilitation (Palmer *et al.*, 2003; Geber & Moeller, 2006; Arceo-Gómez & Ashman, 2011). Differences in the floral orientation and flower colours of these co-flowering *Clarkia* species are similar to the trait patterns that have been observed in communities of *Oxalis* species in South Africa. Flower colour is significantly aggregated in *Oxalis* communities, but differences in other traits including flower size promote pollinator constancy and suggest that facilitation may drive

the co-occurrence of species with similar flower colours (de Jager *et al.*, 2011).

We found patterns of trait variation consistent with different types of character displacement, including both trait divergence and convergence in sympatry relative to allopatry. Character displacement can generate divergence when trait differences minimize negative or increase positive species interaction (Figure 1.1 G-I), or convergence may occur when there is one phenotype associated with competitive dominance (Kraft *et al.*, 2015a; Figure 1.1 G, J, K). The traits that suggest that interactions between *C. unguiculata* and *C. cylindrica* may have promoted their co-occurrence during community assembly also show patterns of variation consistent with character displacement. Out of the eight plant and floral traits measured in this study, we observed divergence between these two species in floral diameter and petal area, and convergence in flowering time where they co-occur versus where they occur alone. These changes are largely driven by earlier flowering of and increases in the floral diameter and petal area of *C. cylindrica* in sympatry (Figure 1.4; Figure 1.5; Table A.9). In addition, two other pairs of species exhibited patterns consistent with character displacement: *C. speciosa* and *C. unguiculata* exhibited divergence in floral diameter and petal area in sympatry, and *C. speciosa* and *C. xantiana* converged in flowering time at two-species communities. Because most of these traits were significantly variable among open-pollinated maternal families, there is likely heritable variation in these traits, which is necessary in order to attribute phenotypic differentiation to character displacement (Losos, 2000). However, to fully rule out alternative explanations for these patterns of trait change in sympatry, future studies would need to demonstrate that these differences between sympatric and allopatric communities are linked to differential use of a limiting resource (pollinators) that is distributed similarly in sympatry and allopatry (Schluter & McPhail, 1992). Furthermore, given that the observed patterns of trait

differences (divergence or convergence) in sympatry compared to allopatry may result from competitive or facilitative interactions (Abrams, 1987; Fox & Vasseur, 2008; Germain *et al.*, 2017; Kooyers *et al.*, 2017), further studies are needed to determine the type of species interaction driving the observed pattern.

Since the concept of character displacement was first introduced (Brown & Wilson, 1956), interest in and support for the idea has fluctuated over time (reviewed in Stuart & Losos, 2013). Our results provide further evidence for three recent developments in the character displacement literature. First, while character displacement was first articulated as a process driven by resource competition among animals, our study contributes to the growing body of evidence for character displacement in plants (reviewed in Beans, 2014; Table 1.1). Second, while character displacement was initially articulated as a pattern of trait divergence in response to resource competition, both theoretical (Abrams, 1987; Fox & Vasseur, 2008; Germain *et al.*, 2017) and empirical (Kooyers *et al.*, 2017) studies have indicated that convergence may also occur in response to species interactions in sympatry when there is one phenotype associated with competitive dominance (Kraft *et al.*, 2015a). The convergence in flowering time observed in sympatry for two pairs of species of *Clarkia* (Figure 1.5) could be the product of competition or facilitation for pollination (Fox & Vasseur, 2008; Sargent & Ackerly, 2008). Because character displacement refers to a change in a species' phenotype in sympatry relative to allopatry that occurs in response to an interspecific interaction, facilitative interactions (e.g. higher pollinator visitation in sympatry) can generate selection that leads to character displacement (e.g. convergence in flowering time in sympatry) (Sargent & Ackerly, 2008). Third, instances where displacement occurs via different characters or in different directions across communities (non-repeatable character evolution; Germain *et al.*, 2017) have not been

considered character displacement in the past, but are consistent with existing definitions of character displacement. This study provides evidence for variation in character displacement patterns across communities that could result from non-repeatable character evolution, which is discussed below.

*The effects of community composition on patterns of in situ phenotypic differentiation*

The second goal of our study was to determine if the species composition of a community can affect patterns of character differentiation. We expected that indirect interactions in complex communities would either amplify or reduce the effects of pairwise interactions (Barraclough, 2015; terHorst *et al.*, 2015), causing variation in patterns consistent with character displacement across communities which could include non-traditional patterns of character displacement (e.g. convergence in sympatry, non-repeatable character evolution; Germain *et al.*, 2017). We found that when trait differences for a pair of species exhibited significant variation across one-, two-, and four-species communities, the trait differences at two- and four-species communities were always significantly different (Figure 1.4 A, Figure 1.4 C, Figure 1.5 A, and Figure 1.5 B). These differences in the trait values of plants at two- and four-species communities could reflect local adaptation to differences in the selective environments in these communities (e.g. Thompson, 2005), as many studies have demonstrated spatial and temporal variability in selection (reviewed in Siepielski *et al.*, 2009, 2011, 2013). However, both traits and fitness need to be measured across these environments in order to demonstrate that these trait differences are the product of different patterns of selection (Wade & Kalisz, 1990; Strauss *et al.*, 2005). Alternatively, these trait differences may not reflect local adaptation to different selective environments if these traits are not the targets of contemporary selection (Sinervo & Basolo, 1996; Aigner, 2006; Caruso *et al.*, 2017), which can be assessed via manipulations of the environment or the trait. Many studies

have manipulated floral traits, including flower colour, size, and the presence or quantity of nectar rewards, to demonstrate pollinator-mediated selection (reviewed in Campbell, 2009), but these traits are likely not under pollinator-mediated selection in all communities. Regardless of the cause of this trait variation, our results highlight the need to consider different types of sympatric communities in future studies, as patterns of character displacement may be non-repeatable across communities (Germain *et al.*, 2017).

Additionally, the results of these comparisons suggest that patterns of character differentiation in more complex communities may not be predictable from patterns in simpler communities (Barracough, 2015; terHorst *et al.*, 2015; Germain *et al.*, 2017). Relative to the phenotypic differences between species at one-species communities, the direction of change in these phenotypic differences varied at two- and four-species communities. In one instance, trait differences were reduced at both two- and four-species communities, demonstrating convergence (Figure 1.5 A), while in another instance, the trait differences were larger at both two- and four-species communities, demonstrating divergence in both types of sympatry (two-species and four-species communities; Figure 1.4 C). Consistent divergence or convergence in a trait across different sympatric communities would occur when the dominant selective agent or agents are constant across communities (Barracough, 2015; Germain *et al.*, 2017). This is likely to occur when a species interaction is specialized or obligate, as these interactions may remain ecologically and evolutionarily significant in different environments (Thompson, 1999; Althoff *et al.*, 2005). For instance, the obligate yucca moth pollinator *Tegeticula cassandra* was the most important determinant of fruit set for the plant *Yucca filamentosa* across communities that varied in the abundance of two florivore species (Althoff *et al.*, 2005). However, specialization can be difficult to assess, as phenotypic specialization does not always reflect ecological specialization

(Waser *et al.*, 1996; Vamosi *et al.*, 2014; Armbruster, 2017).

In two other instances, the pattern of trait change in two-species communities relative to one-species communities differed from the pattern of trait change in four-species communities relative to one-species communities (e.g. Figure 1.4 A and Figure 1.5 B), however these patterns could still result from a form of character displacement (Germain *et al.* 2017). Differences in trait change at two- and four-species communities could result from significant differences in the selective environments at these two types of multi-species communities (e.g. Thompson, 2005). Differences in the selective environments across communities are likely when species interactions are more generalized (Gómez *et al.*, 2009, 2015; Kay & Sargent, 2009) because the same interactions may have different effects on the selective environment in different communities due to the presence of other interacting species (terHorst *et al.*, 2015). For instance, the traits of the generalist plant *Erysimum mediohispanicum* that were under selection in different populations depended on the intensity of local interactions between pollinators and mammalian herbivores (Gómez *et al.*, 2009). In generalized systems, variation in the dominant plant-pollinator interactions may also generate different patterns of selection (Aigner, 2004; Castellanos *et al.*, 2004; Pérez-Barrales *et al.*, 2007; Gómez *et al.*, 2009; but see Sahli & Conner, 2011). For instance, moth-pollinated populations of *Narcissus papyraceus* differed in several floral traits from populations of the same species that are fly-pollinated, which suggests these pollinators have exerted different selective pressures on these populations (Pérez-Barrales *et al.*, 2007), and hummingbirds and bees responded differently to trait manipulations of *Penstemon* species in flight cage experiments (Castellanos *et al.*, 2004). Alternatively, these patterns could reflect multiple phenotypic solutions to the same ecological pressures (non-repeatable character evolution; Germain *et al.* 2017). Non-repeatable character evolution will be common when the

evolutionary trajectories of species that experience the same contemporary selective pressures were shaped by different ancestral conditions, including the occurrence of chance evolutionary events (Losos, 2011). In order to attribute the observed patterns in trait variation to an evolutionary process mediated by interactions with pollinators, future studies of selection in *Clarkia* communities are needed. Estimating selection and quantifying pollinator availability across different communities will help to determine if these patterns are the product of different selective environments or are an example of non-repeatable character evolution, and estimating selection on flowers exposed to certain pollinator species would help to isolate and clarify the selective effects of different species interactions (Sahli & Conner, 2011).

In conclusion, our study explored the potential for ecological and evolutionary processes to shape communities of plants that share pollinators both during community assembly (ecological sorting) and via contemporary evolution (character displacement). While further research is needed in order to attribute the observed patterns to pollinators, we identified patterns consistent with character displacement and patterns of ecological sorting that could stem from pollinator sharing or other abiotic or biotic factors. Furthermore, our results highlight the importance of considering community dynamics themselves (here, the species composition of a community) as one type of factor (in addition to other biotic and abiotic factors) that can affect the outcome of contemporary evolution. The differences observed between plants' traits at two- and four-species communities reinforce the idea that generalizing about the effects of species interactions across types of communities can be difficult. Future studies of plant-pollinator interactions are tasked with the challenge of considering community context as one of a number of factors that shape and are shaped by this critical mutualism.

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## CHAPTER 2

# DIFFERENCES IN FLOWERING PHENOLOGY ARE LIKELY NOT THE PRODUCT OF COMPETITION FOR POLLINATION IN *CLARKIA* COMMUNITIES<sup>2</sup>

### **Abstract**

*Premise of the Research.* Staggered flowering phenologies among co-occurring species have often been interpreted as the product of competition for pollination, but interspecific flowering time differences could result from facilitative, neutral, or competitive interactions. The consequences of interspecific differences in flowering time may vary with the density and species composition of a community.

*Methodology.* To determine if staggered flowering times are adaptive in communities of *Clarkia* (Onagraceae) in the Kern River Canyon (Kern County, CA, USA), we used potted plants to manipulate the flowering time of two species of *Clarkia* (*C. speciosa* and *C. xantiana*) that flower later than two other species of *Clarkia*. To determine if the consequences of flowering time differences change with community context, we conducted this experiment at three types of communities that contained either zero *Clarkia* species (blank communities), the early flowering species, or the early flowering species and one late flowering focal species (mixed communities).

*Pivotal Results.* *Clarkia speciosa* set an equivalent amount of seeds across all community types and both flowering periods. In contrast, seed set of *C. xantiana* was higher during the early flowering period in all community types. Pollinator visitation to *C. speciosa* was highest in early flowering *Clarkia* communities, while visitation to *C. xantiana* did not vary across flowering

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<sup>2</sup> Eisen, K.E., Campbell, D.R., Richards, E., and Geber, M.A. 2019. Differences in flowering phenology are likely not the product of competition for pollination in *Clarkia* communities. *International Journal of Plant Sciences* 180: 974–986.

periods or community types. Across communities and flowering periods, seed set of *C. xantiana* was positively correlated with site floral density.

*Conclusions.* Flowering later is not adaptive for *C. xantiana*, while flowering time may have no effect on the reproductive success of *C. speciosa*. These patterns indicate that later flowering in both species is likely not a result of interspecific competition for pollinators and may result from non-pollinator-mediated selection. In addition, our results indicate that the density of a community can affect the ecological consequences of a flowering strategy, and by extension, the evolution of flowering phenology.

## Introduction

One mechanism that can facilitate the co-occurrence of ecologically similar species is resource partitioning, wherein species experience less competition because they utilize different portions of the available resources in an environment (Hutchinson 1959; MacArthur and Levins 1967; Schoener 1974). A potential example of resource partitioning that has been studied (e.g. Robertson 1895) and debated for over a century (Rathcke and Lacey 1985; Feinsinger 1987; Ollerton and Lack 1992; Fox and Kelly 1993; Ishii and Higashi 2001; Elzinga et al. 2007) occurs when a community of flowering plant species exhibit staggered flowering phenologies. The most commonly invoked explanation for staggered or asynchronous flowering times is that they minimize competition for pollination (reviewed in Rathcke and Lacey 1985; Fenner 1998), which can take the form of direct competition for pollinator visitation, or heterospecific pollen transfer (Pleasants 1980; Feinsinger 1987; Devaux and Lande 2009).

Despite continued interest in the competition for pollination hypothesis, there are two key issues with its prevalence. The first issue relates to other potential explanations for staggered flowering times. For instance, staggered flowering times could also result from sequential mutualism, where a later flowering species benefits from visitation by pollinators that previously visited earlier flowering species (Waser and Real 1979; Geber and Moeller 2006; Kudo 2006; Ogilvie and Thomson 2016). In addition, aggregated flowering times are not always maladaptive. Overlap in flowering may be beneficial if pollinator fidelity is high (McGuire 1993; Kudo 2006), if plants partition pollinators by placing pollen on different parts of pollinators bodies (Macior 1971; Brown and Kodric-Brown 1979; Botes et al. 2008; Huang and Shi 2013) or by releasing pollen at different times of the day (Stone et al. 1998), or if the benefits of a higher visitation rate outweigh the costs of heterospecific pollen transfer (Schemske 1981; Thomson

1982; Ashton et al. 1988; Gross et al. 2000). Beyond interactions with pollinators or plants that share pollinators, other agents of selection may influence flowering phenology including abiotic factors, herbivory, and fruit dispersers (Rathcke and Lacey 1985; Brody 1997; Kudo 2006; Elzinga et al. 2007). Alternatively, variation in phenology across a community could be random (Gleeson 1981; Rabinowitz et al. 1981; Rathcke and Lacey 1985), neutral (Ollerton and Lack 1992), or shaped by phylogenetic constraints (Kochmer and Handel 1986; Johnson 1993; Wright and Calderon 1995; Madeira and Fernandes 1999; Ollerton and Diaz 1999; Lobo et al. 2003). While the pattern of staggered flowering times has been frequently interpreted as the product of competition for pollination, the type of interaction occurring between two species cannot be predicted from their flowering times alone (Thomson 1982), due to the number of processes that can generate staggered flowering times (e.g. phylogeny, random chance, non-pollinator-mediated selection).

The second issue with the prevalence of the competition hypothesis concerns the extent of the evidence for competition as a driver of staggered flowering times. While many studies have inferred competition to be an important driver of community flowering patterns in empirical studies (Mosquin 1971; Heinrich 1975; Heithaus et al. 1975; Pleasants 1980; Lack 1982; Gross and Werner 1983; Armbruster and Herzig 1984; Armbruster 1986; Rathcke 1988; De Jong and Klinkhamer 1991; Ramsey 1995; Gross 1996; Lobo et al. 2003; Aizen and Vázquez 2006; Botes et al. 2008; Landry 2013) and different forms of competition can lead to staggered flowering patterns in a theoretical study (Devaux and Lande 2009), few studies have tested these inferences with manipulative experiments. Of the five published manipulative studies, two found evidence for competition via elevated heterospecific pollen transfer among co-flowering plants (Waser 1978; Campbell 1985), two found no effect of flowering time on reproductive success

(Armbruster and McGuire 1991; McGuire and Armbruster 1991), and one found evidence for both competitive and facilitative interactions (Ha and Ivey 2017). The sparse amount of direct evidence for the role of competition indicates that further manipulative experiments are needed to improve our understanding of this common feature of plant communities.

The fitness consequences of co-occurring with a co-flowering species are likely to be context-dependent, given that community context can affect the relationship between a trait and fitness (Geber and Moeller 2006; Sletvold and Ågren 2014). Properties of flowering plant communities that can affect pollinator visitation and plant reproductive success include species richness and heterospecific plant density, which can be positively (Thomson 1978; Thomson 1981; Thomson 1982) or negatively (Thomson 1982; Brown et al. 2002; Bell et al. 2005; Flanagan et al. 2009) correlated with the reproductive success of a focal species, although the strength of these effects varies (e.g. Feinsinger et al. 1986; Caruso 1999; Wirth et al. 2011). In addition, change in floral density of co-occurring plant species over the course of a flowering season can alter pollinators' foraging preferences (Waser 1978; Campbell and Motten 1985; De Jong and Klinkhamer 1991; Ramsey 1995), such that flowering time may have indirect effects on reproductive success that are mediated by changes in plant density or by changes in pollinator preference. However, only a small number of studies have investigated how species' densities and phenologies interact to affect reproductive success (Kephart 1983; McKinney and Goodell 2011; Ha and Ivey 2017).

Communities of *Clarkia* in the southern foothills of the Sierra Nevada (California, USA) provide an excellent system for testing how species interactions may affect the ecological consequences of flowering time for two reasons. First, the four outcrossing species of *Clarkia* that co-occur in the region flower later (May-June) than most other plants in the region (Lewis

and Lewis 1955; MacSwain et al. 1973; Moeller 2004); species from other genera in the region tend to flower from February to April in response to winter rain (authors pers. obs.). Second, all four species are primarily pollinated by solitary bees, some of which are specialists on the genus (MacSwain et al. 1973; Moeller 2006; Singh 2014). As a result, we expect that the critical species interactions for *Clarkia* in this region are either intraspecific interactions or interspecific interactions with congeneric species.

To examine whether flowering time affected plant reproductive success and how this relationship was affected by the community context, we manipulated the flowering time of the two naturally late flowering *Clarkia* species (*C. speciosa* and *C. xantiana*) in three different types of plant communities. During both the early and late flowering periods, we placed experimental arrays of potted plants at replicates of three types of communities, which enabled us to separate the effects of flowering time from the effects of co-occurring with congeners and with conspecifics. The three community types were: (1) communities that contained no *Clarkia* naturally (blank sites); (2) communities that contained only the early flowering species (early species only sites); (3) communities that contained the early flowering species and the late flowering focal species that was added to the site (mixed sites). We used this design, along with measurements of pollinator visitation rate and local plant density, to address three questions about the adaptive nature of flowering time differences and whether these effects vary with community context:

Q1: Is reproductive success affected by flowering time, community type, or an interaction between these factors?

Q2: Does pollinator visitation rate or site floral density vary across community types or flowering periods?

Q3: Across community types and flowering periods, is reproductive success affected by pollinator visitation rate, site floral density, or an interaction between these factors?

We made the following predictions for each of these questions:

P1: These species flower during the late flowering period in nature, such that we expected greater reproductive success during the late flowering period across all community types.

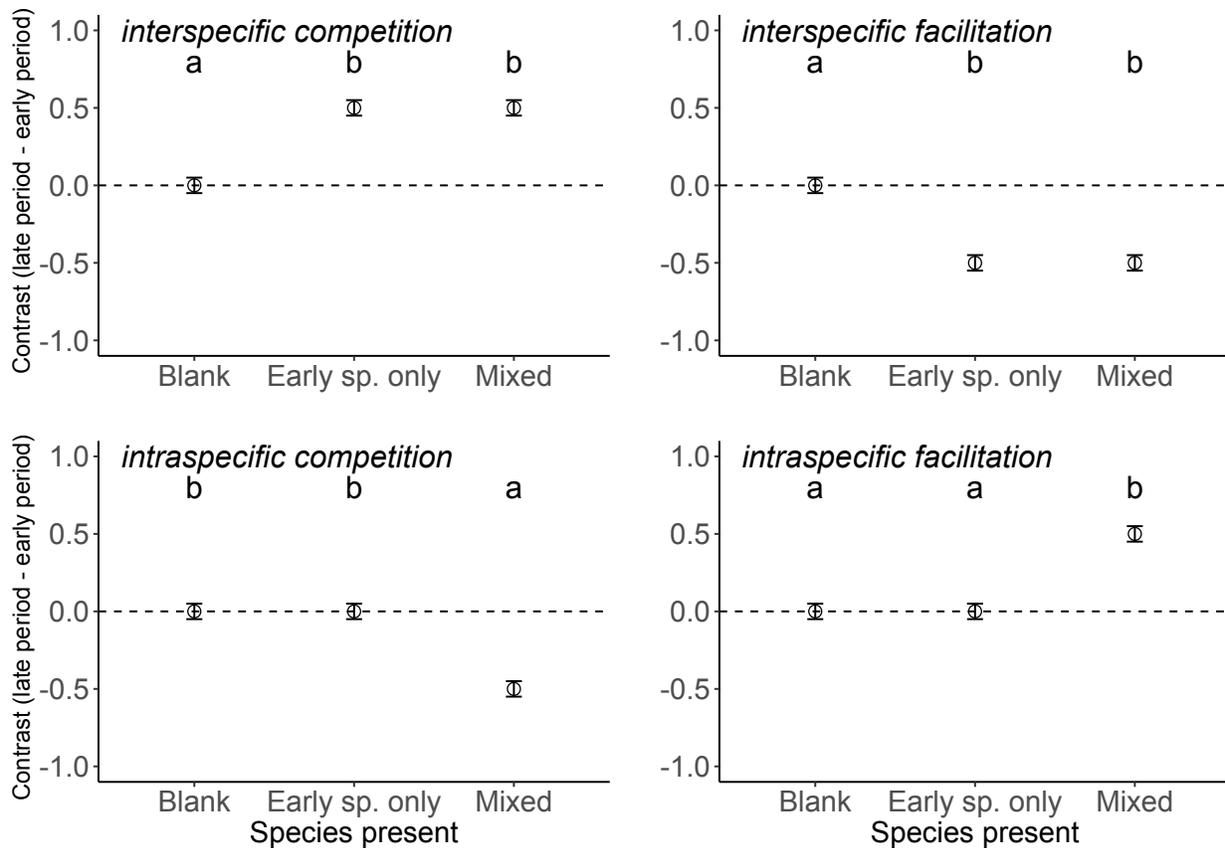
Because greater reproductive success during the late flowering period could be due to the abiotic environment, we used three types of communities in the study to distinguish between the effects of the abiotic environment and species interactions on reproductive success. An interaction between flowering time and the species composition of the experimental community could be driven by relatively stronger interspecific competition or interspecific facilitation with congeners, as well as by intraspecific competition or facilitation, depending on the magnitude and direction of the changes between the flowering periods and across the community types (Figure 2.1).

P2: Because communities in our experiment with naturally occurring *Clarkia* contained either the early flowering species only or the early flowering species and one late flowering species, we expected greater floral density during the early flowering period. We also expected community types with greater floral density to be more attractive to pollinators.

P3: Because pollinators are essential for reproductive success in these *Clarkia* species, we expected greater reproductive success with greater pollinator visitation rate. Local heterospecific plant density could have a positive effect on reproductive success (facilitation) or a negative effect (competition).

## **Methods**

*Study system.* Communities of *Clarkia* species (Onagraceae) provide an ideal system for testing



**Figure 2.1.** Graphical representations of the expected results under four different types of ecological interactions. Within each panel, the community or communities with the larger absolute values of the contrast comparing seed set between the late flowering period and the early flowering period are indicated with the letter ‘b,’ while the community or communities with the smaller differences are indicated with the letter ‘a.’ If the experimental plants experience interspecific competition from the early flowering species (which occur at both early species only and mixed communities), then there will be a positive contrast in seed set at those community types that will be larger than the contrast at blank communities. Conversely, if the experimental plants experience interspecific facilitation from these species, there will be a negative contrast in seed set between the flowering periods in communities that contain *Clarkia* relative to blank communities. If the experimental plants experience intraspecific competition from naturally occurring congeners at mixed communities during the late flowering period, then there will be a larger negative contrast between the flowering periods in mixed communities relative to the other communities. Conversely, if the experimental plants experience intraspecific facilitation from naturally occurring congeners at mixed communities during the late flowering period, then there will be a greater positive difference between the flowering periods in those communities relative to the other communities. Intraspecific interactions and interspecific interactions are not mutually exclusive, although those outcomes are not illustrated in this figure.

whether differences in flowering time minimize competition and promote species co-occurrence. While none of the approximately 40 endemic winter annual plant species in the genus have identical ranges in the Western US, multiple species often occur in sympatry; a community may contain as many as six *Clarkia* species (Lewis 1953). All species of *Clarkia* are annuals, and these communities persist in the same location for long periods—30-40 years or more (Lewis 1953; authors' pers. obs.), creating an opportunity for species interactions to affect the selective environment (Thompson 2005). Within multi-species communities, all *Clarkia* species generally do not flower at the same time (Lewis 1961; Moeller 2004; Singh 2014), and some species exhibit variation in flowering times across their ranges that is not correlated with clinal variation in the abiotic environment (Lewis 1961; Jonas and Geber 1999).

In the Southern Sierra Nevada (Kern River Canyon, Kern County, CA), communities contain up to four outcrossing *Clarkia* species (*C. unguiculata* Lindley, *C. cylindrica* ssp. *clavicarpa* W. Davis, *C. xantiana* ssp. *xantiana* A. Gray, and *C. speciosa* ssp. *polyantha* Harlan Lewis and M. Lewis). Hereafter we refer to each species without its subspecies designation (e.g. *C. xantiana* for *C. x. xantiana*). Multi-species communities are as common as single species communities in the region (Eisen and Geber 2018). The species provide pollen and nectar rewards for bee visitors, and are not known to hybridize in the field (MacSwain et al. 1973). Any selfing in these species is pollinator-mediated because flowers are protandrous and herkogamous: eight anthers mature over 2-3 days before the stigma becomes receptive (Lewis 1953). Outcrossing rates range from 0.79 to 1.0 in *C. unguiculata* (an early flowering species; Vasek 1965; Ivey et al. 2016), and from 0.59 to 0.85 in *C. xantiana* (a late flowering focal species; Moeller et al. 2012; Ivey et al. 2016). The species vary in flowering period and floral orientation: *C. cylindrica* (bowl-shaped flower) and *C. unguiculata* (open-faced flower) flower in

early-mid May, while *C. speciosa* (bowl-shaped flower) and *C. xantiana* (open-faced flower) flower in early-mid June (Moeller 2004; Singh 2014). Most other flowering plants commonly observed in the Kern River Canyon flower in the late winter or early spring (February – April), including *Collinsia heterophylla*, *Eschscholzia californica*, and *Lupinus bicolor* among many other species. Previous work has confirmed that *Clarkia* in the Kern River Canyon share pollinators (MacSwain et al. 1973; Moeller 2006; Singh 2014), which include generalist (polylectic) bees and ten pollen specialists (oligolectic bees) that represent four bee families (Andrenidae, Apidae, Megachilidae, and Melittidae; MacSwain et al. 1973; Moeller 2005).

*Experimental design.* Our experiment tested the effects of staggered flowering times on plant reproductive success. We grew two species of *Clarkia* (*C. speciosa* and *C. xantiana*) from seed in pots over the course of three months to yield plants with a range of flowering times. We used these potted plants to conduct our experiment at three community types: no natural *Clarkia* species present, early flowering *Clarkia* species only, and early and focal *Clarkia* species. We used two or three sites (replicates) for each community type (see Table 2.1). The experiment was conducted at two flowering times (early and late) within each community type for each of the two focal species. As such, the experiment had 3 community types x 2 flowering period x 2 focal species x 2 or 3 sites per community type. During each flowering period, we estimated local plant density in the communities that contained *Clarkia* naturally (see Table 2.1), conducted pollinator observation sessions, and counted the proportion of seeds produced by focal plants to determine how flowering time affects plant reproductive success.

*Experimental set up.* Seeds used for this experiment were collected from eleven sites in June and July, 2015; five of these eleven sites contained both species, resulting in eight collection sites per species (Table B.1). One to several fruits per plant were collected from fifty haphazardly chosen

**Table 2.1.** The sites utilized in the experiment for each focal species. The community types were as follows: mixed communities contained the early flowering *Clarkia* plus the focal species that was experimentally placed at the site, early only communities contained the early flowering *Clarkia* only, and blank communities contained no *Clarkia* present. Thirty plants were placed at each site during each period, such that  $N_{\text{fruit}}$  represents the number of plants that survived the experimental treatment and set one or more fruit(s). Dates and  $N_{\text{fruit}}$  values in italics are sites that were excluded from the analysis due to high levels of herbivory. Two pollinator observation sessions were conducted at each site included in the analysis.

Focal Species	Type	Site name	Latitude	Longitude	Early Period		Late Period	
					Dates	$N_{\text{fruit}}$	Dates	$N_{\text{fruit}}$
<i>C. speciosa</i>	Mixed	CB 279	35.53141	-118.64174	May 14-May 21	30	June 5-June 12	30
	Mixed	Site 30	35.52541	-118.66633	May 17-May 24	29	June 10-June 17	30
	Mixed	Cattle Pens	35.52172	-118.66689	May 20-May 27	28	June 12-June 19	28
	Early only	Summer Camp	35.52943	-118.64599	May 14-May 21	29	June 10-June 17	30
	Early only	Cattle Gate	35.57702	-118.54050	May 17-May 24	26	June 5-June 12	30
	Early only	Demo III	35.53165	-118.62193	May 20-May 27	30	June 12-June 19	30
	Blank	Cattle Pens N	35.52412	-118.66865	May 14-May 21	30	June 10-June 17	27
	Blank	OKCR 28.41	35.56626	-118.57543	<i>May 17-May 21</i>	<i>0</i>		
	Blank	Pizza Place	35.56022	-118.58066	<i>May 20-May 23</i>	<i>0</i>		
	Blank	Borel Jr.	35.59101	-118.51788			<i>June 5-June 9</i>	<i>18</i>
<i>C. xantiana</i>	Mixed	Half Circle Pullout	35.52854	-118.65968	May 11-May 18	30	June 4-June 11	30
	Mixed	Valley of the Gods	35.54549	-118.61692	May 15-May 22	30	June 8-June 15	30
	Mixed	Cow Flat	35.52583	-118.66035	May 19-May 26	30	June 11-June 18	30
	Early only	CB 345	35.56420	-118.58661	May 11-May 18	30	June 4-June 11	30
	Early only	Wet Corner	35.52539	-118.66446	May 19-May 26	30	<i>June 8-June 14</i>	<i>0</i>

(Table 2.1 continues)

Table 2.1 (continued)

Focal Species	Type	Site name	Latitude	Longitude	Early Period		Late Period	
					Dates	$N_{\text{fruit}}$	Dates	$N_{\text{fruit}}$
<i>C. xantiana</i>	Early only	Little Pine	35.57475	-118.54462	May 15-May 20	17		
	Early only	OKCR 25.14	35.55145	-118.60160			June 11-June 18	30
	Blank	CB 372	35.58005	-118.55179	May 12-May 19	29	June 4-June 11	30
	Blank	Dent	35.54998	-118.61614	May 19-May 26	30	June 8-June 15	29
	Blank	Flat Top Rock	35.57558	-118.55678	May 15-May 19	0		

plants of each species at each site.

Seed germination and transplanting took place weekly between January 24-March 27, 2016. Each week, we germinated between 100 and 400 seeds and planted between 50 and 350 seedlings in pots per species as detailed below. During each round of planting, we planted seedlings from each of the eight collection sites for each species. Seeds were placed on moist filter paper in a petri dish, wrapped in parafilm, and stratified at 5°C for seven days. We then transplanted two to three seedlings into 656 mL Deepots™ (D40L, Stuewe & Sons, Tangent, Oregon, USA) filled with Pro-Mix HP Mycorrhizae (Premier Tech Horticulture USA, Quakertown, PA). The plants were grown in randomized positions on benches in a fenced lathhouse at the University of California Irvine Greenhouse, where they were subject to local temperatures and precipitation events which are fairly similar to field conditions. As a result, we watered plants sporadically as needed via misting, showering, or sub-irrigation. We thinned pots after 4-5 weeks to contain one seedling and added 8 prills of Osmocote Flower and Vegetable 14-14-14 fertilizer (The Scotts Company, Marysville, OH).

On April 1-2, 2016, we transported approximately 2,620 plants via truck from Irvine, CA to Lake Isabella, CA. The remaining 360 plants were transported by car on April 10, 2016. In Lake Isabella, plants were maintained at ambient conditions in a fenced enclosure, or in a portable greenhouse structure. This location was over 5 km from the nearest populations of both focal species. We recorded the date of first flower for each plant. For both species, the mean  $\pm$  1 SE number of days between the start of flowering and when a plant was used in the experiment was  $5 \pm 0.25$  days.

*Experimental treatments.* The treatment periods ran from May 11 - May 27 (early period) and from June 4 - June 19 (late period; see Table 2.1). While each treatment at each site lasted one

week, the treatment periods were longer than one week across the three types of communities.

For each focal species, we conducted flowering time treatments at three community types: (1) blank communities, at which no *Clarkia* were present naturally; (2) early flowering communities, which contained the two early flowering species only; (3) mixed flowering communities, which contained the two early flowering species and the late flowering species used as focal species (Table 2.1). Flowering time treatments were replicated at two or three sites per community type for each focal species. Different sites were used for each of the two focal species. The goal was to use the same sites for early and late flowering treatments. In general, we used the same sites for both the early and late flowering period treatments for a focal species. However, two sites used in the early treatment period were not re-used in the late treatment period because of significant herbivory by deer or cattle during the early flowering period; we replaced these sites with other sites of the same community type (Table 2.1). Treatments lasted for seven days (Table 2.1). During the early period, two sites with *C. speciosa* and one site with *C. xantiana* were destroyed by cattle, and one *C. xantiana* site was destroyed by deer herbivory. During the late period, one *C. speciosa* site was destroyed by rabbit herbivory, and one *C. xantiana* site was destroyed by small mammals. These sites were excluded from the analyses during these flowering periods.

We set up two or three sites at a time (Table 2.1). The sites that were set up on a given day contained the same focal species, but differed in community type in order to distribute the replicates of community types across the two week early flowering period and the two week late flowering period. The day before plants were set out, we selected thirty plants per site from all available flowering plants of that species. Each plant included in the experiment had a minimum of three unopened buds at the start of the experimental period to ensure that plants would open

new flowers during the week-long experimental treatment. When selecting plants for each site, we distributed the plants that were flowering from each seed source site across the two or three treatment sites that were set up on the same day, in order to minimize any spurious effects of the source site on plant reproductive success. As a result, each set of thirty plants that comprised an experimental array at a site during a flowering period contained plants from six to eight source sites, although the proportion of plants from each source site varied between arrays conducted during the early and late flowering periods (data not shown).

We recorded the total number of open flowers on each plant at the start of the experimental treatment. To mark which flowers on a plant would be in female phase during the experiment, we tied a piece of green embroidery floss around each stem above any female-phase flowers. (In *Clarkia*, flowering proceeds from the bottom of the stem towards the top, and flowers are in male phase for 2-3 days before they transition to female phase.) To ensure that sites received the same number of open flowers both within and across the treatment periods, we counted the total number of flowers open on all plants in each flowering treatment set. The mean  $\pm$  1 SE total number of open flowers for each *C. speciosa* treatment set was  $80.6 \pm 1.4$ , and the total number for *C. xantiana* was  $119.8 \pm 2.4$ ; these values were determined by the first set of treatment plants for each focal species. If subsequent sets of treatment plants did not fall within this range, we either increased the number of flowers by swapping in plants with more open flowers, or decreased the number of flowers by pinching off open flowers on some plants. We removed any fruits that were beginning to develop on all plants to promote increased investment in flower production.

At the start of the experimental period, we placed the thirty plants at a site into six Deepot™ racks (five plants/rack) in a cluster with no more than 1 meter between each rack. All

thirty experimental plants were placed in a single cluster at each site because *Clarkia* plants tend to occur at high densities (e.g. upwards of 100 flowers per m<sup>2</sup>; KEE unpublished data), and because pollinators may routinely avoid low density patches (e.g. Kunin 1993). In the event that the main stem of a plant broke during transportation and handling, we replaced it with an alternate plant. We used bamboo poles to secure the racks to the hillsides, and we fenced the cluster of plants at each site using plastic deer fencing attached to bamboo poles. Each pot was set in a 9 fl. oz. clear plastic cup, which was filled with water. We refilled cups every 2-3 days.

*Floral density and pollinator visitation rate.* Within the first 1-2 days of each experimental period, we measured the density of naturally occurring *Clarkia* at each site by throwing quadrats along transects and counting the number of stems and open flowers of each species. We threw quadrats every 5 meters along 6-13 transects (mean  $\pm$  1 SE =  $10 \pm 1$  transects) that ran horizontally across the slope of each site. Due to differences in the shapes and sizes of the sites, the shortest transects were 10 meters long and the longest were 125 meters long. Transects were separated vertically (up or down the slope of the site) by 5 meters at smaller sites and by 10 meters at larger sites. This resulted in 46 quadrats at the smallest site and 246 quadrats at the largest site (mean  $\pm$  1 SE =  $93 \pm 16$  quadrats).

To determine if pollinator visitation changed across community types and flowering periods, we conducted two pollinator observation sessions on the experimental plants at each site that was not destroyed by herbivory (see Table 2.1) during both the early and late flowering period. One session took place in the morning (0830-1200) and one in the afternoon (1230-1500); each session lasted for 1 hour. Two observers (KEE and ER) conducted each session. When visitation rates were low to moderate ( $N = 51$  sessions), each observer watched 15 plants in the experimental cluster, and the observers shifted their positions every 15 minutes to reduce

bias and observer fatigue. When visitation rates were high ( $N = 11$  sessions), each observer watched 10 plants at a time, such that there were 10 plants out of the 30 total plants not being observed at any given time. During these sessions, the observers shifted their positions every 10 minutes to watch a different set of plants, which meant that each plant was watched for 40 minutes during the hour-long session. At the end of every session, we counted the total number of open flowers on the experimental plants.

At the end of each week-long experimental treatment, we removed the plants from the field sites and transported them back to the fenced enclosure and portable greenhouse in Lake Isabella, CA. Excluding those sites that were severely damaged by herbivory, the number of plants that survived to produce one or more mature fruits among the sets of 30 plants ranged from  $N = 26$  to  $N = 30$  (see  $N_{\text{fruit}}$  in Table 2.1). To mark which flowers were exposed to pollinators during the experimental treatment, we applied green fabric paint to plants' stems just above the last senesced flower, and we recorded the number of flowers exposed. Plants were not reused in subsequent treatments, and were maintained until they set fruit. When the fruits were mature, we counted the number of mature fruits and collected each mature fruit. The mean  $\pm$  1 SE fruits produced by *C. speciosa* plants was  $5.68 \pm 0.21$  fruits/plant ( $N = 412$  plants), and  $13.83 \pm 0.46$  fruits/plant ( $N = 407$  plants) for *C. xantiana*.

*Data processing.* We used the number of pollinator visits observed during a session and the number of open flowers to calculate a pollinator visitation rate per flower per hour. For the sessions where only two-thirds of the plants were watched at any given time due to high pollinator activity ( $N = 11$ ; see above), we multiplied the number of observed visits by 1.5 and divided by the number of open flowers across all plants to calculate a pollinator visitation rate per flower per hour.

We use the percentage of ovules that set seeds per flower per plant as our measure of reproductive success for two reasons. First, incorporating the number of ovules into the calculation of reproductive success can provide insight into whether a plant is pollen-limited, and facilitates comparisons between fruits that may have produced different numbers of seeds because the fruits had different numbers of ovules. Second, our experimental plants produced more ovules during the early flowering period, likely because plants were at a slightly earlier stage of development (results not shown), and ovaries of earlier flowers contain more ovules. As such, not incorporating ovules into our measure of reproductive success would overestimate the reproductive success of plants in the early treatment. To calculate the average percentage of ovules that set seeds per flower per plant, we counted the number of developed seeds, aborted seeds, and ovules in a maximum of seven fruits per plant. The average proportion of ovules that set seeds per fruit was calculated by dividing the average number of seeds (including developed and aborted seeds) produced by the sum of the average number of seeds and ovules. This proportion was then multiplied by the proportion of flowers that set fruits per plant (the number of fruits divided by the number of flowers) to obtain the average proportion of ovules that set seeds per flower per plant after correcting for fruit set. Data from each of the two focal species were analyzed separately.

*Statistical analyses.* To determine if reproductive success is affected by flowering time, community type, or an interaction between these factors (Q1), we used generalized linear mixed models using the lmer function in the lme4 package in R (Bates et al. 2015). The response variable was the average proportion of seeds per flower (described above). Two random effects were included in all models: the site where the experiment took place and the seed source site. We conducted a model selection procedure that compared the most complex model to all

possible models with fewer terms. The fixed effects in our most complex model were community type (blank, early species only, and mixed), flowering period (early, late), and their interaction, and our least complex model contained only the two random effects described above. We compared AIC values across all models and conducted log-likelihood ratio tests to select the best model, which had the lowest AIC score and also a significant log-likelihood ratio test statistic for a comparison to at least one simpler model. We also used the `r.squaredGLMM` function in the MuMIn package in R (Bartoń 2019) to calculate marginal coefficients of determination ( $R^2_{\text{GLMM}}$ ) for each model; these values represent the amount of variance explained by the fixed effects in each model. For the variables included in the best model, we calculated the estimated marginal means, which are the mean responses for each factor level adjusted for the other variables in the model, for the proportion of seeds set at each level of the flowering period and community type variables and estimated contrasts between flowering periods using `emmeans` function in the `emmeans` R package (Lenth 2018); contrasts between the late and early flowering periods (calculated as late period – early period) at a community type  $\pm$  one standard error are presented.

To determine if pollinator visitation rate or site floral density varies across community types or flowering periods (Q2), we used site-level averages in pollinator visitation rate (visits/flower/hour) and site floral density (flowers/meter<sup>2</sup>). Each of these response variables was examined separately for each focal species using linear mixed models. We conducted model selection using the same methods outlined above. In these models there was only one random effect, the site where the experiment took place, because the data were site-level averages and so the source site data, which was collected on the plant level, could not be incorporated into the analyses. As described above, we used comparisons of AIC values and log-likelihood tests to select the best model, and then we used the estimated marginal means and contrasts across the

factor or factors that were included in the best model to determine the effects of these factors; estimated marginal means  $\pm$  one standard error are presented. Across both flowering periods, higher pollinator visitation at sites with *Clarkia* would be consistent with facilitation, while lower pollinator visitation at sites with *Clarkia* would be consistent with competition.

To determine if pollinator visitation rate, site floral density, or their interaction affect seed set (Q3), we ran additional linear mixed models using the same model structures and model selection procedure used for Q1 (described above).

## Results

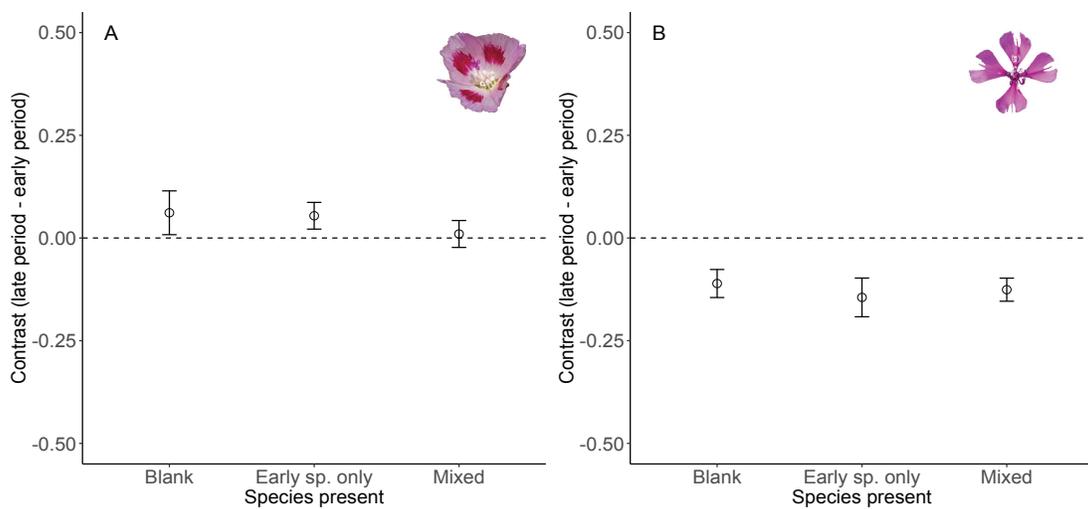
*Q1: Is reproductive success affected by flowering time, community type, or an interaction between these factors?*

*C. speciosa.* None of the models with fixed effects were a better fit than the model that included only random effects of source population and treatment site (Figure 2.2 A; Table B.2).

*C. xantiana.* The best model for the number of seeds per flower of *C. xantiana* was one that included a fixed effect of flowering period ( $\Delta$  AIC = 2.27;  $\chi^2 = 33.121$ ,  $df = 1$ ,  $P < 8.66 \times 10^{-9}$ ; Table B.2). The fixed effect of period explained approximately nine percent of the total variation in seeds per flower (Table B.2). Seed set per flower was consistently higher during the early period relative to the late period at all community types (Figure 2.2 B; Table B.3).

*Q2: Does pollinator visitation rate or site floral density vary across community types or flowering periods?*

*C. speciosa.* For pollinator visitation rate to *C. speciosa*, the model that included a fixed effect of community type was better than the model that included only a random effect of treatment site ( $\Delta$  AIC = 3.297;  $\chi^2 = 7.296$ ,  $df = 2$ ,  $P = 0.026$ ; Table B.4). Pollinator visitation to *C. speciosa* was highest at early species only communities ( $2.697 \pm 0.626$  visits/flower/hour), which is consistent



**Figure 2.2** The contrasts in seed set between the late versus the early flowering period in *C. speciosa* (A) and *C. xantiana* (B) at the three community types (blank—no *Clarkia* present naturally, early sp. only—only the early flowering species (*C. unguiculata* and *C. cylindrica*) present naturally, mixed—the two early flowering species plus the late flowering focal species present naturally). Circles are contrasts calculated as the estimated marginal mean from the late flowering period minus the estimated marginal mean from the early flowering period and error bars are one standard error. In *C. speciosa* (A), seed set did not differ across community types or flowering periods. In *C. xantiana* (B), plants in the early flowering period produced approximately 25 % more seeds than plants in the late flowering period across all community types (negative contrasts indicate greater reproductive success during the early flowering period).

with facilitation between *C. speciosa* and the early flowering species, although differences across community types were not significant (results not shown). For site floral density, none of the models that included fixed effects were better than the model that included only a random effect of treatment site (Table B.5).

*C. xantiana*. For pollinator visitation rate to *C. xantiana*, none of the models with fixed effects were a significant improvement over the model that included only a random effect of treatment site (Table B.4). For site floral density, none of the models that included fixed effects were better than the model that included only a random effect of treatment site (Table B.5).

*Q3: Across community types and flowering periods, is reproductive success affected by pollinator visitation rate, site floral density, or an interaction between these factors?*

*C. speciosa*. None of the models with fixed effects were a better fit than the model that included only random effects of source population and treatment site (Table B.6).

*C. xantiana*. The best model for the number of seeds per flower of *C. xantiana* was one that included a fixed effect of site floral density ( $\Delta$  AIC = 7.27;  $\chi^2 = 9.279$ , df = 1,  $P = 0.002$ ; Table B.6). There was a positive relationship between site floral density and seed set per flower for *C. xantiana* (Figure 2.3).

## **Discussion**

Our study sought to determine whether staggered flowering times are adaptive for two species of *Clarkia* that flower later than co-occurring congeners, and whether the ecological interactions between species with staggered flowering times vary with community context. We observed equivalent seed set for *C. speciosa* across flowering periods and greater seed set for *C. xantiana* during the early flowering period. These results suggest flowering time may not affect the reproductive success of *C. speciosa*, and that late flowering is not adaptive for *C. xantiana*.

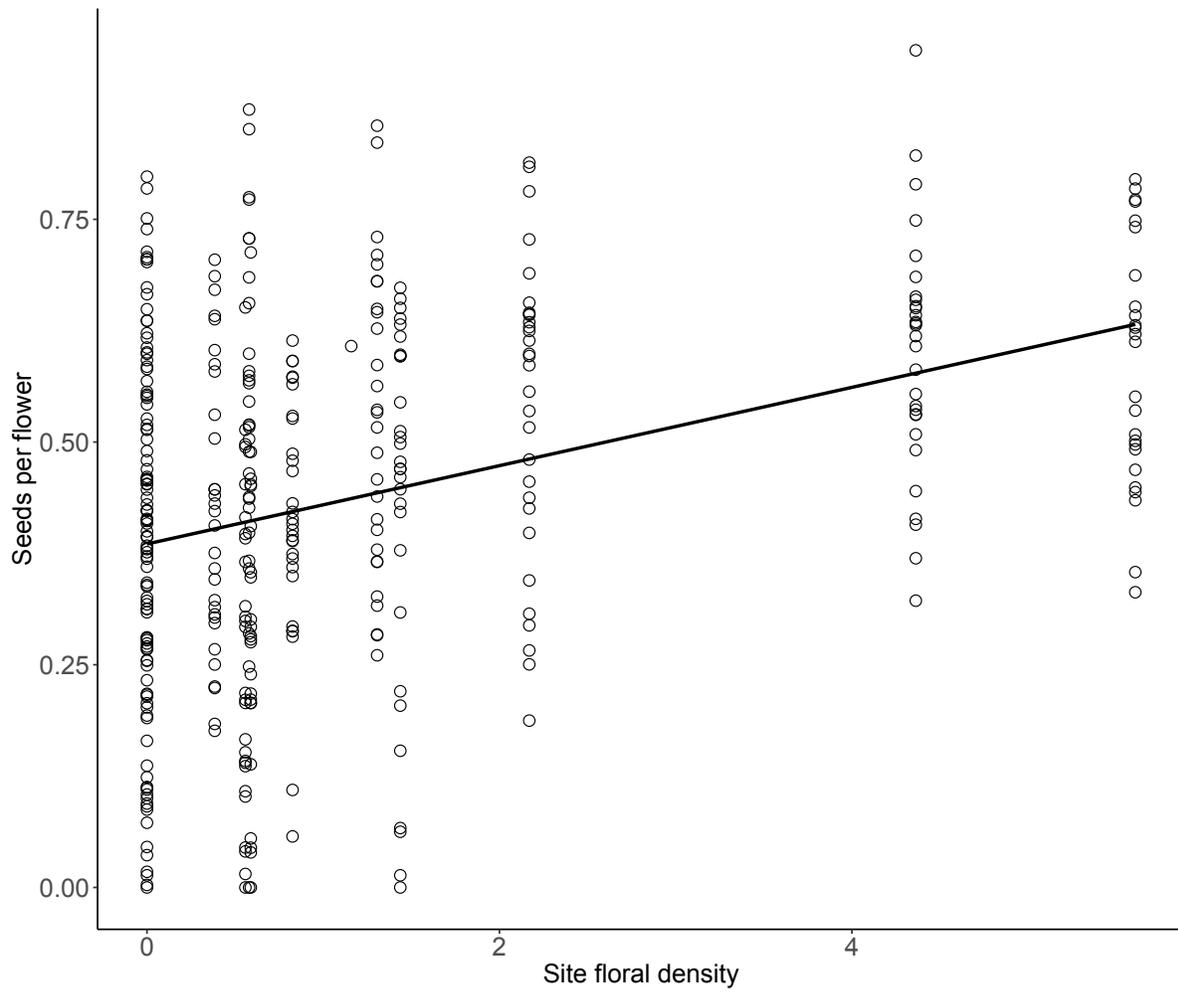


Figure 2.3. Site floral density was positively correlated with seed set per flower in *C. xantiana*.

These patterns indicate that later flowering in both species is not likely to result from interspecific competition for pollination and may result from non-pollinator-mediated selection. In addition we observed a significant effect of one community context factor, floral density, on the reproductive success of *C. xantiana*. Here we discuss what types of ecological interactions and evolutionary processes likely contribute to the observed patterns, and what additional data would be needed to attribute these patterns to specific drivers.

#### *Clarkia speciosa* had equivalent seed set across the flowering periods

Across all communities, seed set of *C. speciosa* did not differ between the flowering periods (contrasts close to or overlapping zero; Figure 2.2 A). These results for *C. speciosa* are similar to those of two studies of co-blooming species in interior Alaska, which also found no effect of synchronous flowering on reproductive success (Armbruster and McGuire 1991; McGuire and Armbruster 1991). In *C. speciosa*, the observed similarity in reproductive success across the flowering periods could result from different types of species interactions in each flowering period. For instance, pollinator visitation rates to *C. speciosa* did not differ between the early and late flowering periods, but previous studies have documented that *Diadasia angusticeps*, an oligolectic bee that is primarily active late in the flowering season (Singh 2014; A.R.M. James unpublished data), is the main pollinator of *C. speciosa* in the region. While this specialized relationship likely contributes to the reproductive success of *C. speciosa* during the late flowering period, equivalent reproductive success during the early flowering period could result from joint attraction of other pollinators that typically visit the early flowering species. Joint pollinator attraction is one form of interspecific facilitation has been observed in a number of systems (Brown and Kodric-Brown 1979; Schemske 1981; Ashton et al. 1988; Gross et al. 2000), including communities of *C. xantiana* (Moeller 2004). Tracking visitation rates by

specific bee species and estimating the single visit efficiency of these pollinators (Sahli and Conner 2007; Ne'Eman et al. 2010) would provide insight into whether different bee species could be equally effective pollinators of *C. speciosa* across the flowering periods (Rafferty and Ives 2012).

*Clarkia xantiana had higher seed set when it flowered early*

Across all communities types included in the experiment, *C. xantiana* set approximately 25 % more seeds during the early flowering period compared to the late flowering period (negative contrasts indicate higher reproductive success during the early flowering period, Figure 2.2 B). This result was not consistent with the expectation that staggered flowering times in multi-species *Clarkia* communities are driven by competition for pollination among co-occurring species. Rather, *C. xantiana* may have experienced relatively stronger facilitation from the early flowering species. Despite the pervasive expectation of competition for pollination, the presence of congeners can be facilitative if pollinators are more attracted to diverse communities (Thomson 1978) and exhibit sufficient constancy in foraging (Thomson 1982). *Clarkia xantiana* represents one of the best documented cases of facilitation due to increased pollinator visitation in multi-species communities (Moeller 2004). However, the significant contrast in reproductive success at blank communities suggests that the early period may offer more favorable abiotic conditions (e.g. De Jong and Klinkhamer 1991).

*Later flowering in both species could result from ecological specialization, developmental constraints, trait correlations, or non-pollinator-mediated selection*

The neutral effect of flowering time on seed set in *C. speciosa* and the positive effect of earlier flowering on seed set in *C. xantiana* suggests that the late flowering observed in nature is largely determined by a factor other than the potential for competition with or facilitation from

the early flowering species. There are four possible explanations for later flowering time, which does have a genetic basis in *C. xantiana* (Gould et al. 2014). First, late flowering could result from ecological specialization on some pollinators which can occur without the evolution of phenotypic specialization that would preclude effective pollination by other pollinators (Aigner 2006; Armbruster 2017). Pollination by multiple species or functional groups of pollinators without apparent fitness trade-offs occurs in a number of generalized systems, including *Dudleya greenei* (Aigner 2004), *Pontederia cordata* (Harder and Barrett 1993), *Erysimum* species (Gómez et al. 2014), and *Impatiens pallida* and *Erythronium grandiflorum* (Wilson and Thomson 1996). Second, late flowering could be due to a developmental constraint (Diggle 1999; Ehrlén 2015). For example, a species of orchid (*Catasetum viridiflavum*) in Panama does not flower in synchrony with its primary pollinator in part due to constraints from factors that affect vegetative growth (Zimmerman et al. 1989). A similar but weaker relationship was observed in an understory forest herb (*Lathyrus vernus*), in that conditions of vegetative growth restrict flowering time but plants with similar vegetative phenologies may vary in flowering phenology (Sola and Ehrlén 2007). Because both *C. xantiana* and *C. speciosa* grown from seed in a greenhouse environment has a longer development time than the early flowering species (Eisen and Geber 2018), the length of their developmental periods may cause these species to flower later than the early flowering species. Third, flowering time in the late flowering species could result from a trait correlation if later flowering is correlated with a trait that is under strong selection (Ehrlén 2015). For instance, multivariate selection analyses of two populations of *Arabidopsis lyrata* indicated that selection on some phenological traits was primarily due to correlated selection on inflorescence number (Sandring et al. 2007). However, this type of correlated evolution may be unlikely in *C. xantiana* due to weak correlations between phenology

and floral or vegetative traits (Dudley et al. 2007; Gould et al. 2014). Fourth, late flowering in *C. xantiana* or *C. speciosa* may be the product of selection from another agent, such as herbivores or seed predators (reviewed in Brody 1997; Ehrlén 2015). Selection from pre-dispersal seed predators tends to favor later flowering (reviewed in Strauss and Whittall 2006; Elzinga et al. 2007), although earlier flowering may be more advantageous in this system due to the existence of other species that may provide forage for herbivores earlier in the spring. Abiotic factors could also serve as agents of selection on flowering time. While earlier flowering in annuals is thought to be adaptive in order to complete the lifecycle before severe heat or drought stress (e.g. Levitt 1980) and could have contributed to higher seed set of *C. xantiana* during the early flowering period, later flowering may be a product of dehydration avoidance, which is another potential strategy for coping with seasonal drought (Ludlow 1989).

*Relationship between site floral density and seed set suggests C. xantiana experiences facilitation from co-occurring congeners*

Across all community types and both flowering periods, we observed a positive effect of floral density on the proportion of seeds set by *C. xantiana*. While high heterospecific density is expected to lead to interspecific competition for pollination (Feinsinger 1987; Ghazoul 2006; Seifan et al. 2014), a positive relationship between total floral density and reproductive success has been observed in a number of systems where co-occurring species contribute to joint pollinator attraction or joint pollinator maintenance (Thomson 1981; Thomson 1982; Johnson et al. 2003; Liao et al. 2011; Ye et al. 2014), including previous studies of *C. xantiana* (Moeller 2004; Moeller and Geber 2005) and other species in the genus (Ha and Ivey 2017). Facilitation mediated by joint pollinator attraction to patches with higher floral density is particularly likely for *C. xantiana* for several reasons. Previous studies have identified a positive relationship

between intraspecific density and pollen deposition (Moeller 2004), increased visitation to *C. xantiana* in communities with co-occurring congeners (Moeller 2004; Moeller and Geber 2005), as well as significant overlap in the primary pollinators of *C. xantiana* and the two early flowering species (Moeller 2005; Singh 2014; A.R.M. James unpublished data). In addition, *C. xantiana* exhibits tolerance to heterospecific pollen transfer (Arceo-Gómez et al. 2016), which could cause the benefits of joint pollinator attraction or joint pollinator maintenance to outweigh any associated interference competition from heterospecific pollen transfer (Thomson 1982). Further work is needed to determine if the early flowering species also experience facilitation. These effects may not be reciprocal for the early flowering species if pollinators display a preference for *C. xantiana* (Brown et al. 2002; Seifan et al. 2014; A.R.M. James unpublished data) or if the early flowering species are not tolerant of heterospecific pollen transfer (Bell et al. 2005; Flanagan et al. 2009).

### *Caveats*

Our study had three limitations. First, due to the logistical challenges associated with manipulating flowering time in natural communities, we conducted our experiment at a small number of sites of each community type for each species ( $N = 2-3$  sites during each flowering period). All previous studies have only manipulated flowering time at one site (Waser 1978; Campbell 1985; Armbruster and McGuire 1991; McGuire and Armbruster 1991). In addition, significant herbivory from cattle and deer further reduced our sample size in three instances (see Table 2.1). Most notably, fruits were only collected from one blank site for *C. speciosa* during the early flowering period. Second, our experimental design consisted of placing 30 potted plants in a cluster at each experimental site. We did this to maximize the signal of these experimental plants, since *Clarkia* often occur at high densities (authors pers. obs.). However, this

experimental design may have significantly decreased the potential for pollen or mate limitation among the experimental plants. Repeating the experiment with experimental plants in a number of smaller clusters within each site rather than one larger cluster may provide more insight into whether flowering time is adaptive in different community contexts. Third, due to the intensive nature of the experiment, we were only able to conduct two pollinator observation sessions during each treatment period at each site. While we avoided conducting observation sessions on cloudy or cool days, variation in climatic conditions in addition to sampling error associated with small sample sizes may have influenced our results. Further innovation in automated methods of conducting pollinator observations (e.g. Weinstein 2015) will improve our ability to quantify pollinator visitation.

### *Conclusions*

Staggered flowering phenologies have been interpreted as the product of competition for pollination for over a century, but our study suggests that flowering time differences may not be adaptive in *Clarkia* communities in the foothills of southern Sierra Nevada. Other factors aside from competition, such as specialized interactions with pollinators, developmental constraints, and trait correlations likely contribute to the staggered flowering phenologies observed. In addition to supporting alternative hypotheses for the existence of staggered flowering times, our results provide indirect evidence of relatively stronger interspecific facilitation in these communities and indicate that flowering density can affect the ecological consequences of a flowering strategy, and by extension, the evolution of flowering time. While manipulating flowering time in the field remains logistically challenging, additional experimental studies that manipulate both flowering time and community context factors will contribute to our understanding of how floral trait evolution occurs in response to selection mediated by

pollinators and other agents.

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## CHAPTER 3

# EMISSION RATES OF SPECIES-SPECIFIC VOLATILES VARY ACROSS COMMUNITIES OF *CLARKIA* SPECIES: EVIDENCE FOR MULTI-MODAL CHARACTER DISPLACEMENT<sup>3</sup>

### **Abstract**

A current frontier of character displacement research is to determine if displacement occurs via multiple phenotypic pathways and varies across communities with different species compositions. Here, we conducted the first test for context-dependent character displacement in multimodal floral signals by analyzing variation in floral scent in a system that exhibits character displacement in flower size, and that has multiple types of sympatric communities. In a greenhouse common garden experiment, we measured quantitative variation in volatile emission rates of the progeny of two species of *Clarkia* from replicated communities that contain one, two, or four *Clarkia* species. The first two axes of a constrained correspondence analysis, which explained 24 percent of the total variation in floral scent, separated the species and community types, respectively. Of the 23 compounds that were significantly correlated with these axes, nine showed patterns consistent with character displacement. Two compounds produced primarily by *C. unguiculata* and two compounds produced primarily by *C. cylindrica* were emitted in higher amounts in sympatry. Character displacement in some volatiles varied across sympatric communities and occurred in parallel with displacement in flower size, demonstrating that this evolutionary process can be context-dependent and may occur through multiple pathways.

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<sup>3</sup> Eisen, K. E., Geber, M. A., and R. A. Raguso. 2020. Emission rates of species-specific volatiles vary across communities of *Clarkia* species: Evidence for multi-modal character displacement. bioRxiv doi: 10.1101/2020.04.02.022004 and in review at *The American Naturalist*.

## Introduction

Interspecific interactions have long been hypothesized to have significant effects on patterns of biodiversity (Darwin 1859; Lack 1945; Schluter 2000; Grant and Grant 2008). One such evolutionary process is character displacement, which leads to a pattern of differences in species' trait values in sympatric communities relative to allopatric communities (Brown and Wilson 1956; Germain et al. 2017). While character displacement has been studied and debated for over sixty years (Stuart and Losos 2013), there are two key gaps in our understanding of this process. First, outside of a small number of classic systems (e.g., anoles, sticklebacks, Darwin's finches), few studies have examined the potential for character displacement in more than one type of trait (reviewed in Stuart and Losos 2013). Examining variation in multiple types of traits increases our ability to detect non-repeatable character displacement, which may occur through different phenotypic pathways across communities (Losos 2011; Germain et al. 2017), and determine when species interactions lead to shifts in correlated or independently-evolving traits. Second, while most studies of character displacement have focused on pairwise interactions (but see Lemmon and Lemmon 2010; Miller et al. 2014a; Grant 2017; Roth-Monzon et al. 2020), many species exist in complex ecological communities, where interactions with multiple species could include indirect and higher-order interactions (Mayfield and Stouffer 2017; TerHorst et al. 2018; Roth-Monzon et al. 2020). Testing for character displacement across sympatric communities that vary in species composition or richness (Eisen and Geber 2018; Roth-Monzon et al. 2020) can advance our understanding of the evolutionary consequences of direct and indirect interactions (Walsh 2013; TerHorst et al. 2015).

Among co-occurring flowering plants, pollinators often represent a shared resource that is critical for reproduction (Waser et al. 1996; Ollerton et al. 2011), and there is a growing body of

evidence for character displacement in plants mediated by interactions between co-occurring species that share pollinators (reviewed in Beans 2014; Eisen and Geber 2018). Presently, there are two critical gaps in our understanding of this process. First, studies to date have examined character displacement in floral morphology, color, and phenology (reviewed in Beans 2014; Eisen and Geber 2018), which reflects a general bias towards visual traits in pollination (Raguso 2008a). Nonetheless, olfactory and reward traits are critical to successful pollination in many systems (Schiestl 2010, 2015; Raguso 2014) but have yet to be integrated in to the study of character displacement. Second, character displacement in floral traits is likely to occur via multiple phenotypic pathways or changes in trait combinations across communities (Losos 2011; Germain et al. 2017). Nonetheless, most studies to date have addressed character displacement in one type of floral trait (e.g., color or morphology), not the multi-modal bouquet flowers typically present (Leonard et al. 2011).

The emission of floral scent—volatile organic compounds including monoterpenes, sesquiterpenes, and aromatic compounds (Knudsen et al. 2006)—is a complex trait, in that individual plants can exhibit qualitative variation in the blend of volatiles and quantitative variation in their emission rates (Raguso 2008b). Because scent can be produced not only from petals but also from reproductive floral structures, scent may be correlated with or unrelated to variation in flower size (Effmert et al. 2006; Burdon et al. 2015; Martin et al. 2017), which could lead to multi-modal character displacement in some systems. In addition, species may vary in common volatiles that are produced by a small number of biosynthetic pathways (Dudareva and Pichersky 2006), and in species-specific volatiles that provide ‘private channels’ for communication with specialist pollinators (Raguso 2008b; Soler et al. 2010). Insights from three areas of floral scent research suggest that floral scent could undergo character displacement.

First, floral scent exhibits substantial intraspecific variation across populations in multiple systems, including cacti (Schlumpberger and Raguso 2008), cycads (Suinyuy et al. 2012), saxifrages (Friberg et al. 2019), and orchids (Gross et al. 2016; Chapurlat et al. 2018). These patterns suggest that floral scent may be relatively evolutionarily labile. As a result, scent could evolve in response to geographic variation in selection (Gross et al. 2016), which could lead to variation in character displacement across different communities (Germain et al. 2017; Eisen and Geber 2018). Second, floral scent can be a target of pollinator-mediated selection (Parachnowitsch et al. 2012; Chapurlat et al. 2019), which indicates that floral scent could evolve in response to interactions between co-occurring plant species that share pollinators. Third, differences in floral scent can mediate reproductive isolation between co-occurring species (Waelti et al. 2008; Bischoff et al. 2014; Peakall and Whitehead 2014) and explain variation in the structure of plant-pollinator networks (Junker et al. 2010; Larue et al. 2016; Kantsa et al. 2018, 2019). As such, floral scent may determine how pollinators are partitioned among co-occurring plant species.

In this study, we test for variation in multimodal character displacement across sympatric communities that contain different numbers of co-occurring species. Specifically, we assess the potential for character displacement in the floral scent of two co-occurring species of California native annuals in the genus *Clarkia* (Onagraceae). These species, *C. unguiculata* and *C. cylindrica*, co-occur more frequently than expected by chance in the southern foothills of the Sierra Nevada (Kern County, CA, USA). Where they co-occur, these species have converged in flowering time and diverged in flower size (Eisen and Geber 2018). This pattern of divergence in flower size provides an opportunity to test if character displacement occurs on multimodal floral signals. We conducted a greenhouse common garden experiment to measure quantitative

variation in volatile emission rates of the progeny of plants from natural communities that contain one, two, or four *Clarkia* species. By eliminating variable environmental effects on trait values, the common garden enabled us to compare phenotypes across different, replicated community types and test for significant interactions between species and community types on floral volatile emission rates. These data were used to address three questions regarding the potential for and nature of character displacement in floral scent:

(1): Is variation in volatile emissions across species and community types consistent with character displacement?

(2): Do patterns of character displacement vary across types of sympatric communities? (e.g., two-species vs. four-species communities)

(3): Do multi-modal signals (e.g., floral scent and flower size) jointly undergo character displacement?

## **Methods**

*Study system.* Species in the genus *Clarkia* (Onagraceae) often co-occur and share pollinators, which are primarily solitary bee pollinators that specialize on the genus (Lewis 1953; MacSwain et al. 1973; Singh 2014). Across the genus, species exhibit intra- and interspecific variation in multiple types of floral traits, including flowering time (Lewis 1961; Jonas and Geber 1999; Moeller 2004; Gould et al. 2014; Singh 2014), floral orientation (Lewis 1961), petal coloration (Lewis and Lewis 1955), flower size (Eisen and Geber 2018), and floral scent (Miller et al. 2014b). In the Southern Sierra Nevada (Kern River Canyon, Kern County, CA), *C. unguiculata* Lindley and *C. cylindrica* ssp. *clavicaarpa* W. Davis co-occur more frequently than expected by chance (Eisen and Geber 2018). These species are primarily outcrossing because flowers are protandrous and herkogamous, and while they share pollinators (Singh 2014), they are not

known to hybridize in the field (MacSwain et al. 1973). The petal area of *C. cylindrica* exhibits divergent character displacement (an increase in petal area) relative to *C. unguiculata* in communities that contain two- and four-species of *Clarkia* (Eisen and Geber 2018).

*Common garden source community selection.* Our common garden contained three replicates of each of four unique types of source communities: *C. cylindrica* alone, *C. unguiculata* alone, *C. cylindrica* and *C. unguiculata* together, and these two species with the two other outcrossing *Clarkia* species (*C. speciosa* and *C. xantiana*) that occur in the Kern River Canyon (for community locations, see Table C.1). In other words, individuals of each species (*C. cylindrica* and *C. unguiculata*) were grown from seeds sourced from three single-species communities, three two-species communities, and three four-species communities. Community types thus vary in how many species are present in the community. Seeds from both species were collected at all communities in 2017. Three or more fruits per plant were collected from 50 haphazardly chosen plants of each species at each community. The seeds from one fruit from each of 20 plants per community and species were combined to ensure that plants in the common garden represented a sufficient range of any possible plant-level variation at each community.

*Plant germination and growth.* Because of the large number of community x species combinations present in the common garden, seeds were started in five batches in September-November 2017. All community x species combinations were included in each batch of plants. To break dormancy, seeds were placed on moist filter paper in a petri dish, wrapped in parafilm, and stratified at 5°C for five to seven days and then held at 23°C for five to seven days before planting. Germinants were transplanted into 656 ml<sup>3</sup> Cone-tainers (Stuewe & Sons, Tangent, Oregon, USA) filled with Lambert soil mix. The pots' positions on the greenhouse bench were randomized. Plants were exposed to supplemental light (16 h days) and maintained at 23-25°C

during the day and 19-21°C at night. Plants were watered twice a week on average and received on average 30-40 mL of water per week in weeks 1-3 post transplanting, 70-80 mL per week in weeks 4-6, and 100 mL per week in weeks 7-10. Each pot initially contained two germinants; pots were thinned after four weeks to contain one plant. At this time, six prills of Osmocote® Smart-Release® Plant Food Flower & Vegetable 14-14-14 fertilizer (Scotts Miracle-Gro Company, Marysville, OH) were applied to the soil surface in each pot.

*Qualitative scent analysis.* To inform our quantitative sampling protocols, we conducted two types of qualitative analyses using Solid Phase Micro Extraction (SPME) fibers (Supelco, Inc., (Sigma-Aldrich), Bellefonte, PA) (Appendix C-1). First, to determine if the presence of additional flowers changed the composition of the volatile profile (i.e., due to threshold dosage effects), we compared the profiles of samples with three versus six cut flowers from the same plant. We recovered significantly more monoterpenoid and sesquiterpenoid compounds in samples with six flowers (Appendix C-1). Given this result, we adjusted our quantitative headspace sampling protocol to include a minimum of six open flowers per plant (see below). Second, to determine where volatiles are produced in these flowers, we compared the volatile profiles of dissected petals from six flowers versus those of the remaining tissues of the same six flowers. We found that the petal and non-petal samples separated in multivariate space (Figure C.1). Petals generally contained fewer volatiles than the non-petal floral tissues (Appendix C-1), which may influence the relationship between flower size and floral scent (see Discussion).

*Quantitative scent analysis.* Floral volatile samples were collected using the dynamic headspace adsorption technique between November 13, 2017, and February 5, 2018. All collections were made under natural lighting conditions in a well-aerated glassed-in corridor adjacent to the greenhouse where the plants were grown. We used an AIRCARE hygrometer (Essick Air

Products, Little Rock, AR, USA) to record the minimum and maximum temperature and percent humidity during sampling; the average minimums were 17 °C and 23 percent humidity, while the average maximums were 25 °C and 41 percent humidity. Floral samples were obtained from fifteen plants per community per species ( $N_{total} = 270$ ), and one vegetative control sample was collected per community per species ( $N_{total} = 18$ ).

We used 16 oz PET water bottles to enclose stems for headspace sampling. Water bottles were washed with odorless soap, dried, and baked in a clean drying oven for 15-20 mins at 80 °C each morning before sampling began. Samples were collected using PAS-500 Micro Air Sampler pumps (Spectrex, Inc., Redwood City, CA) connected to traps that contained 0.0100 g of Tenax 80/100 adsorbent (Alltech Associates, Inc. (W.H. Grace), Deerfield, IL, USA). The flow rate of the pump was set to 200 mL/min. Scent was collected for 6 hours, from 900 to 1500, as this corresponds to the period of greatest pollinator activity in natural communities. For floral samples, 6-13 flowers were enclosed per plant (average: 7.4 flowers), and the number of flowers enclosed was recorded for each plant sample. Vegetative controls were obtained from plants that had not begun to flower but had formed buds. During each sampling day, one ambient control sample was collected in an empty PET bottle.

Immediately following the end of the headspace collection period, the traps were removed from the pumps and eluted with 300  $\mu$ L of GC-MS quality hexane (Burdick & Jackson GC2; Honeywell International, Inc. USA). Samples were then concentrated to 50  $\mu$ L with a flow of gaseous N<sub>2</sub>, and spiked with 23 ng of toluene (5 mL of a 0.03% solution in hexane) as an internal standard in preparation for analysis with gas chromatography - mass spectrometry (GC-MS; see below). Samples were stored at -20C and labeled with a community-neutral identifier code based on the date of sampling (e.g., December 15-1, December 15-2, etc.) to facilitate

analysis that would be blind to the species and community type (see Becklin et al. 2011).

*Scent analysis via GC-MS.* Both solvent-eluted and solvent-free (SPME) volatile samples were analyzed using a GC17A gas chromatograph coupled with a QP5000 quadrupole mass spectrometer (Shimadzu Scientific Instruments, Inc., Kyoto, Japan). One  $\mu\text{L}$  aliquots of the solvent eluted samples were injected (splitless mode) at 240C onto a polar GC column (EC Wax, 30m long, 0.25 mm internal diameter, 0.25 $\mu$  film thickness; BGB Analytik). The GC oven program (40C to 240C, increasing at 20C per minute, with a 2-minute hold at the maximum temperature) was optimized to minimize run length (for over 300 samples) while allowing for peak resolution to baseline. Electrical ionization mass spectra were generated under 70eV conditions (scanning range 40-350 m/z), and resulting mass spectra were compared with those of MS libraries (Wiley, NIST, Adams) using Shimadzu GCMSolutions software. Kovats retention indices (KI) were prepared for each compound by running a blend of n-alkanes (C7-C30) under the same chromatographic conditions and optimized method. Volatile compounds were identified via 1) direct comparison of retention time and mass spectra with those of authentic standards, 2) comparison with the KI of the best MS library fit for the unknown with published KI values from the plant volatile literature (NIST WebBook; <https://webbook.nist.gov/>), or 3) in the absence of standards or published KI values, the mass spectral data (ion fragment table) were listed for unknown compounds in reverse order of abundance, starting with the base peak (set to 100%).

*Extraction and processing of quantitative data.*

Peak areas were integrated manually using Shimadzu GCMSolutions software. After excluding compounds that were present in one or two samples out of 270, our quantitative dataset contained 54 compounds (Table C.2). To exclude experimental artifacts from individual

plants' profiles, we compared the profile of each sample to the profile of the ambient control that was collected on the same day. If a compound appeared in both a floral sample and the relevant ambient control, we only retained this peak in the floral sample if the floral sample peak area was at minimum five times larger than the peak area of the ambient control. This value was selected to be highly conservative in terms of the compounds that we retained in samples where those compounds were also present in the control. Similarly, to exclude compounds emitted by vegetation, we compared each floral sample to the vegetative control collected from the sample population and applied the same 5x threshold to any overlapping compounds. As such, some compounds were retained in the dataset but excluded from particular samples where their emission rates were similar to the relevant ambient or vegetative controls.

Emission rates were normalized by dividing total ion current (TIC) peak areas by that of the internal standard (Svensson et al. 2005), then were calculated algebraically using response factors generated using external standard dose-response curves generated from log- and semi-log dilutions of the primary floral volatiles identified in these analyses (*(E)*- $\beta$ -ocimene,  $\alpha$ -pinene,  $\beta$ -caryophyllene, benzyl alcohol, and methyl salicylate).

To relate emission rates to floral masses, we measured the fresh and dry masses of twenty flowers (ten male-phase and ten female-phase flowers) per community and species (Table C.1). Flowers were selected haphazardly from between four and eight plants per community and species. Each plant contributed a maximum of five flowers to the 20 total flowers per community and species. Fresh masses were recorded immediately after removing the flower from the plant. Flowers were dried for 24 hrs at 50 °C before dry masses were recorded. We present analyses of floral scent emission rates that were standardized by the number of open flowers that contributed to a sample multiplied by the average fresh mass of a flower from that community and species,

which gives the  $\mu\text{g}$  scent per g fresh floral mass per hr. Analyzing the data using emission rates that were standardized by the number of open flowers that contributed to a sample ( $\mu\text{g}$  scent per flower per hr) yielded highly similar results (results not shown).

*Additional common garden to test for wounding artifacts.* We observed differences across species and community types in compounds that are generally considered “green leafy volatiles” (abbreviated as GLVs) and are associated with plant wounding (Visser and Ave 1978; Scala et al. 2013) (see Results). To determine if these patterns resulted from artifacts of the experimental sampling process, we conducted an additional common garden experiment to test for differences in the emission rates of GLVs between wounded and non-wounded plants (Appendix C-2). Wounding elevated the emission rates of GLVs (e.g., (Z)-3-hexen-1-ol, (Z)-3-hexenyl acetate; Table C.3), but the emission rates of these compounds during the 2018 main experiment were more similar to the 2019 non-wounded control plants than to the 2019 wounded plants (Table C.4). As such, observed emission rates of these compounds are unlikely to be an experimental artifact and we retain the GLVs as floral compounds in our analysis.

*Multivariate statistical analyses and dimensionality reduction.* We used multivariate and univariate methods to test for significant interactions between species and community type, which provides evidence for differences in species' emission rates of volatiles across communities (character displacement). All analyses were performed in R (R Core Team 2018). To partition the observed variance in the emission rate of all compounds across the species and community types, we performed a Permutational Multivariate Analysis of Variance (PERMANOVA) using a Bray-Curtis distance matrix using the *adonis* function from the *vegan* package (Oksanen et al. 2018). We specified our replicate communities nested within community type as strata in the function, which is equivalent to a random effect. To find the compounds that

distinguish the community types and species, we performed a Canonical Analysis of Principal Coordinates with a Bray-Curtis distance dissimilarity index using the `capscale` function from the `vegan` package (Oksanen et al. 2018).

*Univariate statistical analyses.* We analyzed variation in compound classes and in specific compounds using general linear mixed-effects models, which were performed using the `lme4` package (Bates et al. 2015). Models were assessed to ensure normally distributed residuals with homogenous variance. These models all contained community type, species, and their interaction as fixed effects, and community nested within community type was included as a random effect. The significance of fixed effects in linear mixed models was assessed using the ANOVA function in the `lmerTest` package ver. 2.0-29 (Kuznetsova et al. 2015) to perform type III F tests using the Kenward-Roger approximation for the denominator degrees of freedom. When ANOVAs returned significant F values, we used Tukey's honest significant difference tests to determine which group means were significantly different using the `emmeans` function with the `pairwise` option in the `emmeans` package (Lenth 2019). These tests were performed with the `'type = "response"'` option such that intervals were back-transformed from the log and square-root scales. Contrasts for models with log-transformed response variables are presented on the log-odds scale, such that ratios greater than one indicate larger emission rates in *C. cylindrica* and ratios less than one indicate larger emission rates in *C. unguiculata*.

We performed two types of univariate analyses. First, we tested for differences in total scent emission and the emission of certain types of compounds across the species and community types using linear mixed-effects models as described above. The compound classes we analyzed were monoterpenoids, sesquiterpenoids, GLVs, and aromatics (Table C.2). To ensure that our models had normally distributed residuals with homogenous variance, total scent,

monoterpenoid, and aromatic emission rates were square-root transformed and GLVs and sesquiterpenoid emission rates were log-transformed.

Second, we performed univariate analyses on compounds that were correlated with one or both of the first two CAP axes. Specifically, 23 compounds were correlated with one or both of the first two CAP axes (Table C.5). These compounds had significant Pearson correlation coefficients with one or both axes at  $P < 0.01$  after applying a false discovery rate correction for 54 tests; all significant correlations were greater than 0.15 or less than -0.15. Most compounds were either square-root or log-transformed to improve the normality of model residuals (Table C.5). To test for a significant interaction between species and community type, we ran ANOVAs on these models as described above. We applied a false discovery rate correction for 23 tests on the  $P$  values associated with these species by community type interactions. We then tested for differences between species and community types using the emmeans function as described above.

## Results

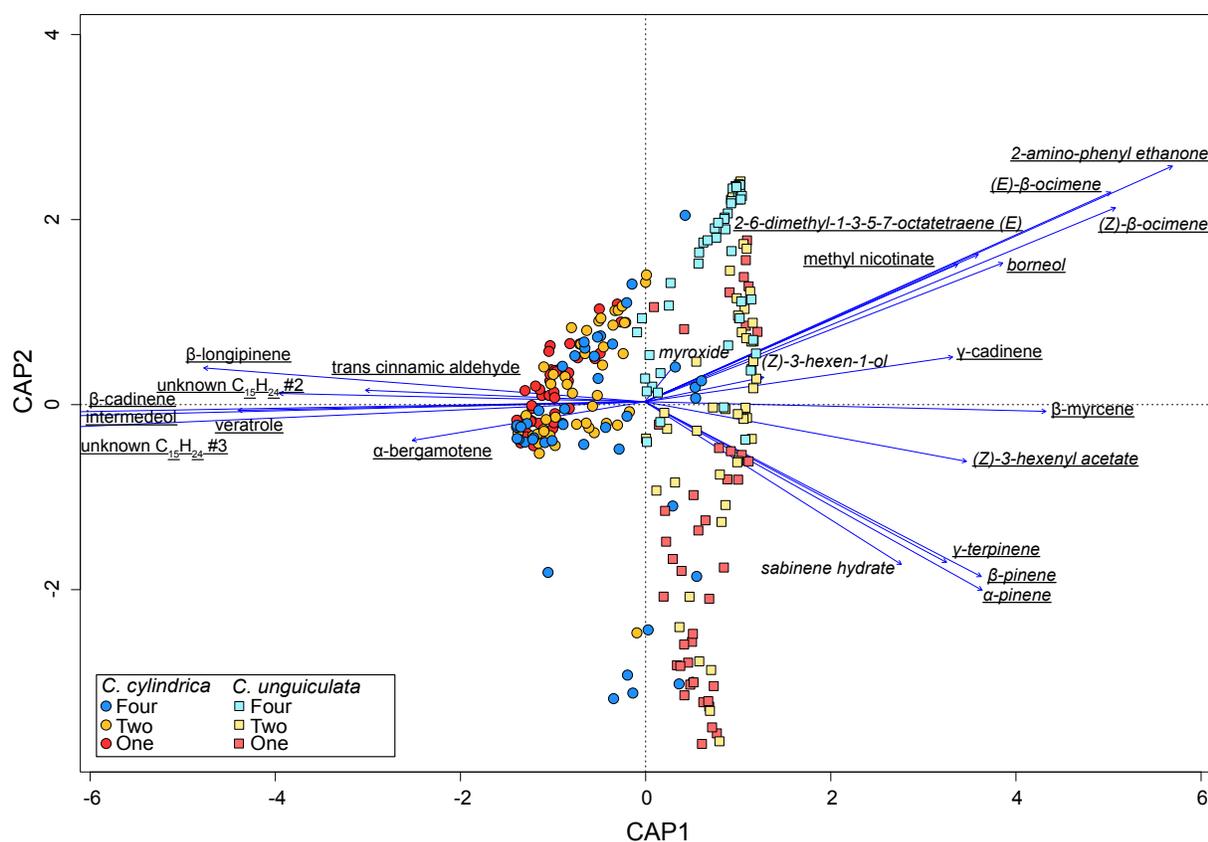
*Diversity of volatile organic compounds.* There were 54 volatile organic compounds present in four or more samples: 22 monoterpenoids, 18 sesquiterpenes and C<sub>15</sub> derivatives, five GLVs and nine aromatic or nitrogenous compounds (Table C.2). Thirty-eight of the 54 compounds were found in more than five samples of both species. Of the remaining 16 compounds, 11 compounds were completely or nearly unique to *C. unguiculata* (present in five or fewer *C. cylindrica* samples), and five compounds were completely or nearly unique to *C. cylindrica* (present in five or fewer *C. unguiculata* samples). The average number of compounds detected in a sample (mean  $\pm$  1 SE) was  $13.8 \pm 0.4$  for *C. cylindrica* and  $14.0 \pm 0.5$  for *C. unguiculata*.

*Multivariate analyses.* The PERMANOVA on the scent compounds revealed main effects of

community type ( $R^2 = 0.03$ ,  $P < 0.001$ ), species ( $R^2 = 0.22$ ;  $P < 0.001$ ), and an interaction between the two ( $R^2 = 0.04$ ,  $P < 0.001$ ).

The Canonical Analysis of Principal Coordinates indicated that a subset of all compounds helped to define variation among the six species x community type combinations in our study (two focal species x three community types per species). The constrained portion of the variance was 24% of the total variance (our independent variables explained 24 % of the total variation in the data). CAP axis 1 explained 20 % of the total variation in scent and 77 % of the constrained variance. In general, *C. cylindrica* individuals had negative values on CAP axis 1, while *C. unguiculata* individuals had positive values (Figure 3.1, Table C.6). CAP axis 1 was strongly positively correlated with two monoterpenoids and an aromatic compound, and strongly negatively correlated with sesquiterpenoids (Table 3.1, Table C.5). CAP axis 2 primarily separated *C. unguiculata* individuals from the three different community types (Table C.6). This axis explained 4 % of the total variation in scent, and 14 % of the constrained variance. It was strongly positively correlated with two monoterpenoids and an aromatic compound, and strongly negatively correlated with two GLVs and a monoterpenoid (Table 3.1, Table C.5).

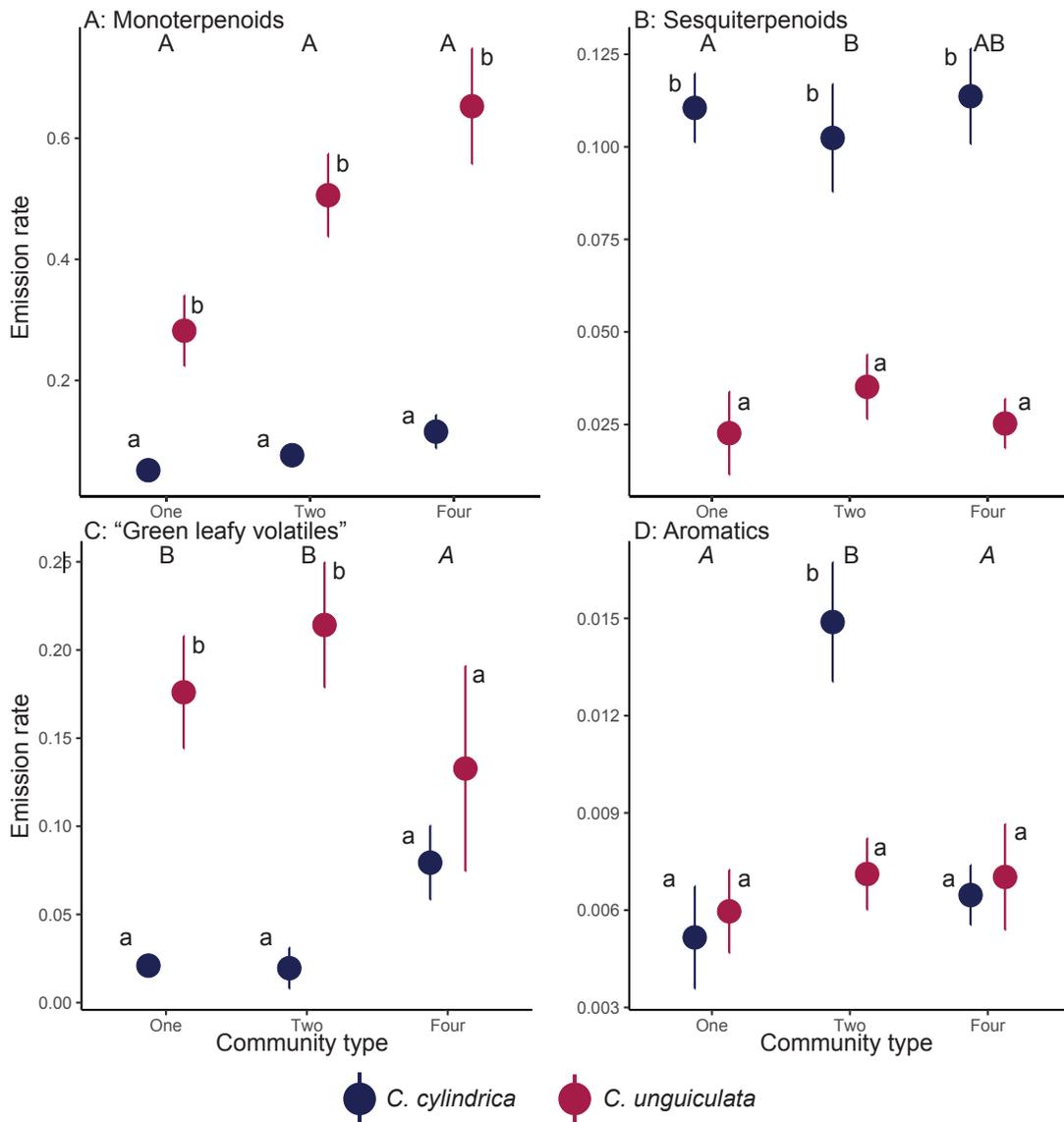
*Univariate analyses of total scent, compound classes, and single compounds.* Patterns of variation in three compound classes were consistent with character displacement: sesquiterpenes ( $F_{2,117.84} = 8.749$ ,  $P = 0.0003$ ), GLVs ( $F_{2,78.42} = 17.330$ ,  $P < 0.0001$ ), and aromatics ( $F_{2,89.51} = 4.720$ ,  $P = 0.0113$ ). The interaction in sesquiterpene emissions was driven by a significantly larger difference between the species in one-species communities relative to two-species communities (Figure 3.2; Table C.7). For the GLVs, *Clarkia unguiculata* produced more than *C. cylindrica* at one-species communities and two-species communities; the interaction was driven by both species producing equivalent amounts of these compounds at four-species



**Figure 3.1. Canonical analysis of principle coordinates separating volatile emissions from the two species and three community types. The different species and community type combinations are indicated by color and symbol type. The loadings are shown with arrows for compounds with the largest positive and negative correlations ( $r$ ) with CAP axis 1 (underlined compounds), and for compounds with the largest positive and negative correlations ( $r$ ) with CAP axis 2 (italicized compounds). CAP axis 1 strongly separates the species, as *C. cylindrica* have lower values and *C. unguiculata* have higher values. CAP axis 2 primarily differentiates the community types of *C. unguiculata*, as individuals from one-species communities had generally low values (centroid= -1.212), individuals from two-species communities had intermediate values (centroid: -0.061), and individuals from four-species communities had high values (centroid: 1.330).**

Table 3.1. The three compounds with the strongest positive and negative correlations with the first two CAP axes. For CAP1, nine additional compounds (two “green leafy volatiles,” five monoterpenoids, one sesquiterpenoid, and one aromatic compound) were significantly positively correlated with this axis, and three additional sesquiterpenoids and two aromatic compounds exhibited significant negative correlations ( $r > 0.15$ ; Table S3). For CAP2, two additional monoterpenoids and one aromatic compound were also positively correlated with this axis, and three additional monoterpenes exhibited significant negative correlations ( $r > 0.15$ ; Table C.5). A false discovery rate correction for conducting 54 tests was applied to all  $P$  values (see Methods).

<b>Axis and compound</b>	<b><i>r</i></b>	<b><i>P</i></b>
<b>CAP1</b>		
( <i>Z</i> )- $\beta$ -ocimene	0.528	$1.51 \times 10^{-19}$
( <i>E</i> )- $\beta$ -ocimene	0.526	$1.70 \times 10^{-19}$
2-amino phenyl ethanone	0.481	$4.60 \times 10^{-16}$
intermedeol	-0.646	$1.73 \times 10^{-31}$
$\beta$ -cadinene	-0.641	$2.87 \times 10^{-31}$
unknown C <sub>15</sub> H <sub>24</sub> #3	-0.516	$1.04 \times 10^{-18}$
<b>CAP2</b>		
( <i>E</i> )- $\beta$ -ocimene	0.457	$1.26 \times 10^{-13}$
( <i>Z</i> )- $\beta$ -ocimene	0.444	$5.16 \times 10^{-13}$
( <i>E</i> )-2-6-dimethyl-1,3,5,7- octatetraene	0.329	$4.12 \times 10^{-7}$
( <i>Z</i> )-3-hexenyl acetate	-0.349	$6.44 \times 10^{-8}$
( <i>Z</i> )-3-hexen-1-ol	-0.275	$3.52 \times 10^{-5}$
sabinene hydrate	-0.275	$3.52 \times 10^{-5}$



**Figure 3.2.** Emission rates (raw values;  $\mu\text{g}$  scent/g fresh floral mass/hour) of monoterpenoids (A), sesquiterpenoids (B), “green leafy volatiles” (C), and aromatic compounds (D) by *C. cylindrica* (light pink) and *C. unguiculata* (dark pink). Lowercase letters above each point indicate whether emission rates differed between the species within that community type; the letter b indicates the species with the higher emission rate. Uppercase letters above each set of points at a community type indicate whether that difference is the same or different from the differences at other community types; differences with the letter A in italics are not significantly different from zero, and differences with the letters B or C are larger than differences with the letter A. Emission rates of monoterpenoids were higher in *C. unguiculata* (A). *Clarkia cylindrica* produced more sesquiterpenes than *C. unguiculata* at all community types (B), but the difference between the species was significantly larger at one-species communities than at two-species communities. *Clarkia unguiculata* produced more “green leafy volatiles” than *C. cylindrica* at one-species communities and at two-species communities, but emission rates at four-species communities were equivalent (C). *Clarkia cylindrica* had substantially higher emission rates of aromatic compounds at two-species communities (D). Note the differences in scale for the y-axes across all panels.

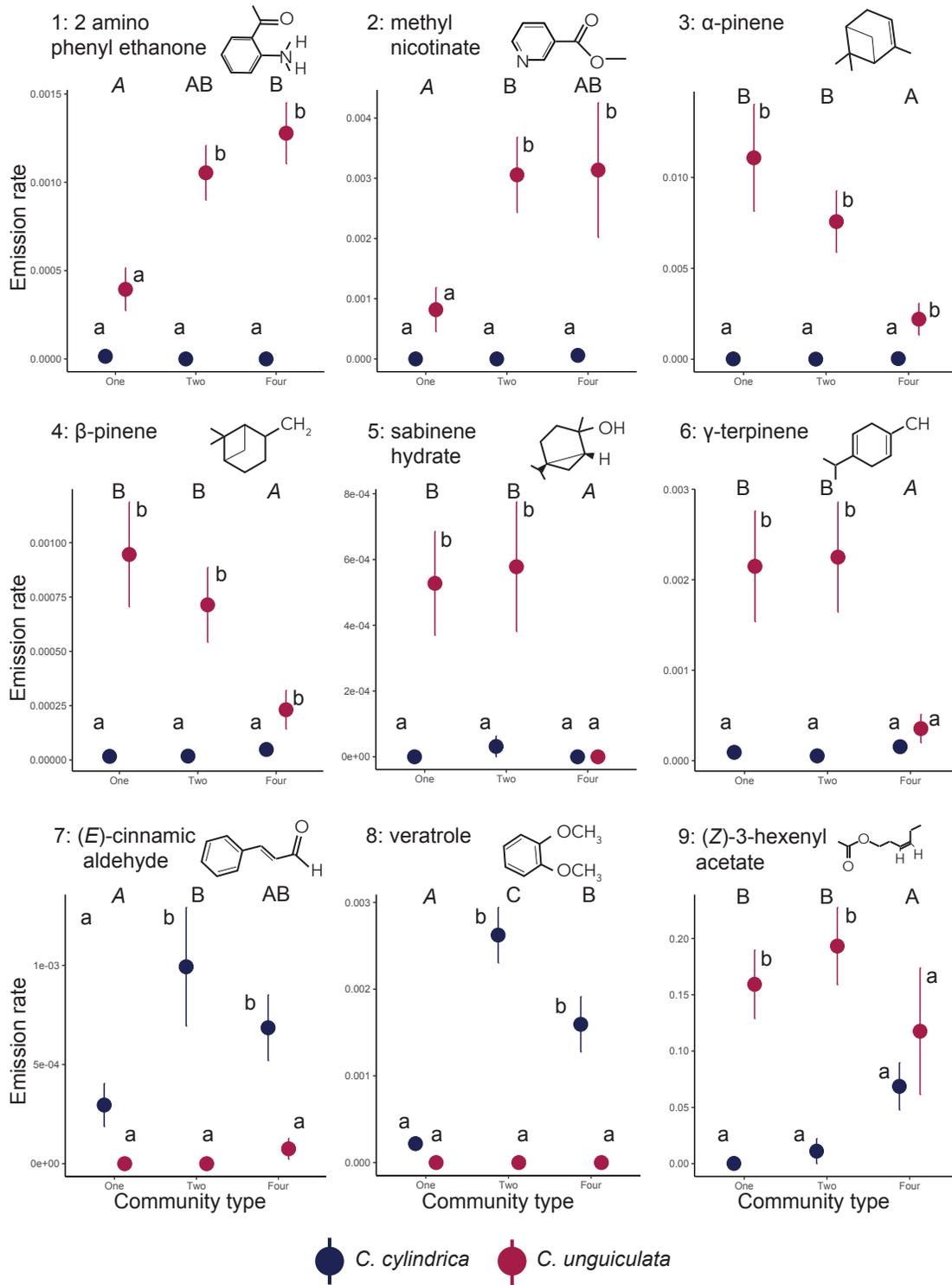
communities (Figure 3.2; Table C.7). The interaction in aromatics emissions was driven by greater production by *C. cylindrica* at two-species communities (Figure 3.2; Table C.7), such that the difference between the species at two-species communities was significantly larger than the differences between the species at one- and four-species communities (Figure 3.2; Table C.7). In particular, this pattern was driven by the emission of large amounts of benzyl alcohol by *C. cylindrica* in two-species communities (results not shown).

We used the results of the Canonical Analysis of Principal Coordinates to determine the compounds that we analyzed individually. Of the 23 compounds that were correlated with one or both of the first two CAP axes (see Methods), nine compounds had significant community type by species interactions in univariate models (Table C.8). Two of these compounds, 2-amino phenyl ethanone, and methyl nicotinate, are primarily produced by *C. unguiculata*, and emission rates were higher at both types of sympatric communities (Figure 3.3 A&B, Table C.7). Two additional compounds, (*E*)-cinnamic aldehyde and veratrole, are primarily or exclusively produced by *C. cylindrica*, and emission rates were higher at both types of sympatric communities (Figure 3.3 G & H, Table C.7). The remaining five compounds with significant interactions,  $\alpha$ -pinene,  $\beta$ -pinene, sabinene hydrate,  $\gamma$ -terpinene, and (*Z*)-3-hexenyl acetate, are primarily produced by *C. unguiculata* and had lower emission rates in four-species communities relative to one- and two-species communities (Figure 3.3, Table C.7).

## **Discussion**

By measuring floral scent variation across communities that contain different numbers of species in a system that exhibits character displacement in flower size, we conducted the first test for context-dependent multi-modal character displacement in floral traits. These species exhibit pronounced differences in their floral scent profiles, with more subtle but significant differences

Figure 3.3 Emission rates (raw values;  $\mu\text{g}$  scent/g fresh floral mass/hour) of the nine compounds that were significantly correlated with one or both of the first two CAP axes and also had a significant species  $\times$  community type interaction. Lowercase letters above each point indicate whether emission rates differed between the species within that community type; the letter b indicates the species with the higher emission rate. Uppercase letters above each set of points at a community type indicate whether that difference is the same or different from the differences at other community types; differences with the letter A in italics are not significantly different from zero, and differences with the letters B or C are larger than differences with the letter A. 2-amino-phenyl ethenone (A) and methyl nicotinate (B) were primarily emitted by *C. unguiculata* (dark pink) and increased in two- and four-species communities.  $\alpha$ -pinene (C),  $\beta$ -pinene (D), sabinene hydrate (E), and  $\gamma$ -terpinene (F) were also primarily emitted by *C. unguiculata* and decreased in four-species communities. (*E*)-cinnamic aldehyde (G) and veratrole (H) were exclusively emitted by *C. cylindrica* (light pink) and the emission rates of these compounds increased in two- and four-species communities. (*Z*)-3-hexenyl acetate (I) was emitted at higher rates by *C. unguiculata* at one- and two-species communities, and by *C. cylindrica* at four-species communities. Note the differences in scale for the y-axes across all panels.



across the community types. In an analysis of all of the volatile organic compounds emitted by the two species, the significant interaction between species and community type was driven by compounds that were primarily or exclusively emitted by only one species—two aromatic compounds and four monoterpenoids emitted by *C. unguiculata*, and two aromatic compounds emitted by *C. cylindrica*. These patterns were consistent across sympatric communities for *C. cylindrica* but not for *C. unguiculata*. In addition, our investigation of the potential for multi-modal character displacement revealed that changes in floral scent were associated with changes in flower size in *C. cylindrica* but not in *C. unguiculata*. Here we discuss the potential drivers and ecological implications of these patterns.

*Character displacement driven by changes in species-specific volatiles.* Because floral scent is a complex trait, character displacement could occur through several pathways, including both qualitative or quantitative changes in compounds that are either shared across the species or unique to one species. In this study, we observed patterns consistent with character displacement in compounds that were generally emitted by only one of the focal species. These types of changes could be linked to increases in plant-pollinator specialization in multi-species communities. Specifically, an increase in species-specific volatile emissions may increase a pollinator's ability to differentiate between two co-occurring plant species, which could increase pollinator constancy and decrease heterospecific pollen transfer among species that share pollinators (Waser 1986; Sargent and Ackerly 2008). Divergence in flower color has been demonstrated to reduce inconstant foraging in multiple systems (Levin 1985; Hopkins and Rausher 2012; Muchhala et al. 2014; Norton et al. 2015). The compounds that exhibited patterns consistent with character displacement in *Clarkia* were benzenoid aromatics (both species) and monoterpenoids (*C. unguiculata*). Among plants that are pollinated by food-seeking bees, scent

profiles are commonly dominated by benzenoids, terpenoids, or a mixture of the two types of compounds (Dobson 2006). In both observational and experimental studies, benzenoids have been associated with visitation from apid and halictid bees (Theis 2006; Andrews et al. 2007; Kantsa et al. 2019), such that the increases in benzenoid emission rates in *Clarkia* could result in greater attraction of these pollinator species. In particular, because only *C. unguiculata* receives upwards of five percent of all pollinator visits from apid bees (*Apis mellifera*, *Xylocopa tabaniformis*, *Bombus* sp.; Singh 2014), the increases in benzenoid emissions could reflect greater pollinator specialization in sympatric communities.

*Context dependency of character displacement.* Because indirect interactions can modify evolutionary trajectories (Benkman 2013; Walsh 2013; TerHorst et al. 2015), we tested for variation in character displacement in two types of sympatric communities: two-species communities that contain the focal species of this study, and four-species communities that contain the focal species plus two additional congeners that flower later in the summer (Moeller 2004). We found that *C. cylindrica* exhibited similar patterns across both types of sympatric communities, while patterns for *C. unguiculata* across the community types varied by compound class (monoterpenoids and aromatics). In general, this variation in the patterns observed for our two focal species points to the potential for character displacement to be context-dependent (Eisen and Geber 2018; Roth-Monzon et al. 2020) and to occur via different phenotypic pathways across communities (Germain et al. 2017). In particular, our results suggest that changes in the volatile profile of *C. cylindrica* may result primarily from interactions with *C. unguiculata*, which occurs in both types of sympatric communities. For *C. cylindrica*, indirect interactions with the later-flowering *Clarkia* species in the four-species communities may not affect the evolution of floral scent.

In contrast, *C. unguiculata* had greater emission rates of two aromatic compounds at both types of sympatric communities, but lower emission rates of four monoterpenoids only at the four-species sympatric communities. Similar patterns of intermediate or less displacement were observed across different multispecies communities of freshwater fish (Roth-Monzon et al. 2020), which suggests that evolution in these communities likely occurs in response to multiple species interactions. Because *C. unguiculata* is the earliest *Clarkia* species to flower in the region (Moeller 2004; Singh 2014), its higher total scent emission (see Figure 3.3) may serve to attract scarce pollinators at the beginning of the flowering season (Filella et al. 2013). However, the observed decrease in monoterpenoid emissions in the four-species communities suggests that *C. unguiculata* may invest less in pollinator attraction if, like other species of *Clarkia* (Moeller 2004), it experiences facilitation in these communities.

*Multi-modal character displacement: synergy of changes in floral size & scent.* Because pollinators often exhibit responses to combinations of visual and olfactory traits (Leonard et al. 2011), we conducted the first test for character displacement in multi-modal floral signals (Figure 3.4). Using estimates of volatile emission rates that were standardized by floral fresh mass, we found that changes in the floral scent of *C. unguiculata* were not associated with changes in flower size, while increases in the emission of floral scent of *C. cylindrica* were related to increases in flower size. We hypothesize that this pattern results from differences in the floral parts that produce these compounds (Effmert et al. 2006). Our floral dissections suggest that the character displacement compounds in *C. cylindrica* are produced in both the petals and the reproductive parts, which is consistent with a correlation between flower size and floral scent. In contrast, the character displacement compounds in *C. unguiculata* were present more often in the reproductive parts relative to the petals (Appendix C-1), which is consistent with a

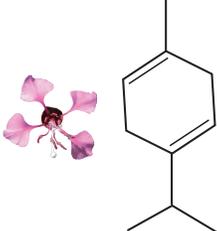
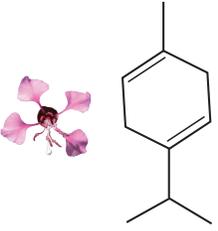
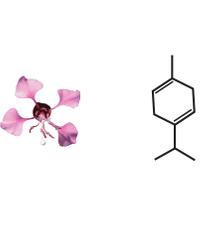
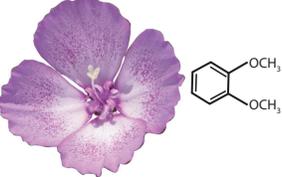
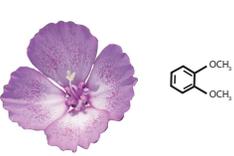
	One-species communities	Two-species communities	Four-species communities
<i>C. unguiculata</i>			
<i>C. cylindrica</i>			

Figure 3.4. Schematic showing the relative changes in flower size (based on measurements of petal area in Eisen & Geber 2018) and the species-specific floral scent compounds that showed patterns consistent with character displacement (*C. unguiculata*: 2-amino phenyl ethenone,  $\alpha$ -pinene,  $\beta$ -pinene, sabinene hydrate,  $\gamma$ -terpinene, and methyl nicotinate; *C. cylindrica*: (*E*)-cinnamic aldehyde and veratrole). Drawings of flowers and chemical compounds (molecules that are representative of the suites of compounds that responded in each species) are scaled proportionally both between the species and across the community types. Flower size of *C. unguiculata* is similar across community types and is slightly smaller than the flower size of *C. cylindrica* at one-species communities. Floral scent emission rates of *C. unguiculata* are similar at one- and two-species communities, and emission rates at four-species communities are about 0.45 times emission rates at one-species communities (a decrease in scent emission in four-species communities). Emission rates of floral scent in *C. unguiculata* are one or two orders of magnitude higher than emission rates in *C. cylindrica*. Flower size of *C. cylindrica* at two-species communities is 1.7 times larger than flower size at one-species communities, and flower size at four-species communities is 1.25 times larger than at one-species communities. Floral scent emission rates of *C. cylindrica* are 7.2 times larger at two-species communities relative to one-species communities, and 4.6 times larger at four-species communities relative to one-species communities.

change in floral volatiles that was independent of a change in flower size. The differences in these patterns highlight that the complexity of floral scent can extend beyond the quantitative and qualitative composition of a scent bouquet to include spatial variation in the emission of volatiles across tissue types (Friberg et al. 2013; Burdon et al. 2015; Martin et al. 2017).

These differences in the floral sources of the volatiles that change across the community types may signify differences in their functions. For *C. cylindrica*, the increases in both size and volatile emissions in both petals and reproductive parts may serve to increase overall pollinator attraction in sympatry. Increases in flower size or scent emission have been linked to increased pollinator attraction and plant reproductive success in multiple insect-pollinated systems (Conner and Rush 1996; Miyake and Yafuso 2003; Majetic et al. 2009; Sandring and Ågren 2009; Parachnowitsch et al. 2012), although most studies have not tested for concurrent changes in both traits (but see Parachnowitsch et al. 2012). For *C. unguiculata*, scent emission in the reproductive tissues may serve to cue pollinators to the precise location of the reproductive parts (Dötterl and Jürgens 2005; Burdon et al. 2015). Because the solitary bees that specialize on *Clarkia* forage for pollen (MacSwain et al. 1973), volatiles emitted in the reproductive tissues also indicate the location of the primary rewards for this species. After becoming attracted to a flower, bees can use pollen odors, which are often a distinct subset of the floral bouquet (Jürgens and Dötterl 2004; Effmert et al. 2006), to orient more specifically to the source of pollen (Dobson et al. 1996, 1999). Here, the decrease in floral volatiles that are putatively produced in the reproductive organs in four-species communities suggests that *C. unguiculata* may invest less not only in pollinator attraction as described above, but more specifically in provisioning pollinators with pollen where the community of congeners may facilitate joint pollinator attraction or maintenance (Moeller 2004). This hypothesis could be tested via additional analysis

of the pollen volatiles in *C. unguiculata*, and with pollinator behavior assays (see below).

*Future directions.* This study yielded a pattern of trait variation that is consistent with character displacement, but additional work is needed to rule out alternative hypotheses (Schluter and McPhail 1992). In particular, it is critical to determine if this variation in scent has functional consequences for pollinator behavior. Given that the volatiles that mediate pollinator behavior are often a subset of all volatiles emitted by a plant (reviewed in Junker and Blüthgen 2010), pollinators may not respond to the specific changes observed in floral scent profiles across community types. Experimental assays of pollinator behavior can be used to determine if these shifts in volatiles affect pollinator attraction or constancy, or if they are non-functional. The potential effects of variation in *C. unguiculata* volatiles on honey bees and bumblebees could be tested in a controlled environment (e.g., Burger et al. 2012; Peter and Johnson 2014). However, a comprehensive assessment of the functionality of floral scent variation in *Clarkia* would need to be field-based, as lab experiments with the solitary bees that pollinate both species are not tractable.

More broadly, the results of this study highlight the need to continue to integrate chemical phenotypes into the study of floral trait evolution (Leonard et al. 2011; Junker and Parachnowitsch 2015). In combination with visual traits, floral scent can affect species interactions at multiple scales, from specifying highly specialized interactions (e.g., Peakall and Whitehead 2014; Whitehead et al. 2015) to contributing to the structure of complex plant-pollinator interaction networks (Kantsa et al. 2018, 2019). Systems that exhibit variation in both floral scent and species interactions across communities (e.g., Friberg et al. 2019) provide opportunities to study the interplay between complex trait evolution and species interactions, which can generate insight into the repeatability of evolutionary change across variable

ecological communities.

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## CHAPTER 4

### FLORAL DENSITY AND CO-OCCURRING CONGENERS ALTER PATTERNS OF SELECTION IN ANNUAL PLANT COMMUNITIES<sup>4</sup>

#### **Abstract**

While the evolution and diversification of flowers is often attributed to pollinator-mediated selection, interactions between co-occurring plant species can alter patterns of selection mediated by pollinators and other agents. The extent to which both floral density and congeneric species richness affect patterns of net and pollinator-mediated selection on multiple co-occurring species in a community is unknown and is likely to depend on whether co-occurring plants experience competition or facilitation for reproduction. We conducted an observational study of selection on four species of *Clarkia* (Onagraceae) and tested for pollinator-mediated selection on two *Clarkia* species in communities differing in congeneric species richness and local floral density. When selection varied with community context, selection was generally stronger in communities with fewer species, where local conspecific floral density was higher, and where local heterospecific floral density was lower. These patterns suggest that intraspecific competition at high densities and interspecific competition at low densities may affect the evolution of floral traits. However, selection on floral traits was not pollinator-mediated in *C. cylindrica* or *C. xantiana*, despite variation in pollinator visitation and the extent of pollen limitation across communities for *C. cylindrica*. As such, interactions between co-occurring species may alter patterns of selection mediated by abiotic agents of selection.

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<sup>4</sup> Eisen, K.E., Wruck, A.C., and Geber, M.A. 2020. Floral density and co-occurring congeners alter patterns of selection in annual plant communities. *Evolution* doi: 10.1111/evo.13960

## Introduction

While pollinator-mediated selection is the most commonly invoked explanation for the evolution and diversification of flowers, floral traits are frequently shaped by selection from other abiotic and biotic factors (reviewed in Strauss and Whittall 2006; Caruso et al. 2019). One potential factor contributing to variation in selection through space and time are the interactions between co-occurring plants in a community. Because species interactions can alter the availability of abiotic (e.g. soil water availability) and biotic (e.g. pollinators) resources, community context factors including conspecific and heterospecific density and the presence of co-flowering species in a community affect pollinator visitation and plant fitness in many systems (studies in plain text in Table 4.1). These interactions between co-occurring species should modify the opportunity for selection (Benkman 2013; Vanhoenacker et al. 2013), and have variable effects on patterns of selection, given that interactions can range from facilitative to competitive both within species and among species (Rathcke 1983; Moragues and Traveset 2005; Muñoz and Cavieres 2008; Seifan et al. 2014).

For both intraspecific and interspecific interactions, selection will be stronger at low densities if there is facilitation, or at high densities if there is competition (Table 4.1). These predictions stem from the expectation that positive interactions will not be strong enough to reduce variation in reproductive success at low densities, while strong competitive interactions at high densities will increase variation in reproductive success (Rathcke 1983). In both cases, the existence of variation in reproductive success is expected to create a greater opportunity for selection and lead to stronger selection at these densities (Benkman 2013; Vanhoenacker et al. 2013). In communities that contain more species, selection may be weaker if there is facilitation that decreases variance in reproductive success and thus decreases the opportunity for selection

Table 4.1. Expectations for how variation in plant density and co-occurring species in a community will affect selection on floral traits. For each community property, the different potential conditions and their expected effects on pollinator visitation and selection are presented alongside theoretical (T), empirical (E), and review (R) papers that generated or tested these expectations. References in bold estimated patterns of selection using Lande and Arnold (1983) selection gradient analysis methods. References in plain text measured the effects of these factors on pollinator visitation rates and/or plant fitness but did not estimate selection. Full citations for references are given in Appendix D-1.

<b>Community property</b>	<b>Conditions</b>	<b>Effects on pollinator visitation</b>	<b>Effects on selection</b>	<b>References</b>
Conspecific plant density	Intraspecific facilitation	Higher visitation at higher densities	Stronger selection at low densities	Thomson 1978 <sup>E</sup> , 1981 <sup>E</sup> , Kunin 1993 <sup>E</sup> , 1997 <sup>R</sup> ; Steven et al. 2003 <sup>E</sup> ; Knight 2003 <sup>E</sup> ; Moeller 2004 <sup>E</sup> ; Ghazoul 2005 <sup>R</sup> ; Gunton and Kunin 2009 <sup>E</sup> ; Hegland et al. 2009 <sup>E</sup> ; Dauber et al. 2010 <sup>E</sup> ; Sun et al. 2010 <sup>E</sup> ; Lázaro and Totland 2010 <sup>E</sup> ; Essenberg 2012 <sup>E, T</sup>
	Intraspecific competition	Higher visitation at low densities	Stronger selection at high densities	Rathcke 1983 <sup>T</sup> ; Goulson 2000 <sup>T</sup> ; Steven et al. 2003 <sup>E</sup> ; Campbell and Husband 2007 <sup>E</sup> ; Gunton and Kunin 2009 <sup>E</sup> ; Spigler and Chang 2009 <sup>E</sup> ; Wirth et al. 2011 <sup>E</sup>
			No effect	Schmitt 1983 <sup>E</sup> ; Feinsinger et al. 1986 <sup>E</sup> ; Caruso 1999 <sup>E</sup> , <b>2002<sup>E</sup></b> ; <b>Donohue et al. 2000<sup>E</sup></b> ; <b>Stanton et al. 2004<sup>E</sup></b> ; Sun et al. 2010 <sup>E</sup> ; <b>Weber and Kolb 2013<sup>E</sup></b>
Hetero-specific plant density	Interspecific facilitation	Higher visitation to focal species at high heterospecific densities	Stronger selection at low densities	Schemske 1981 <sup>E</sup> ; Thomson 1982 <sup>E</sup> ; Kunin 1993 <sup>E</sup> ; Moeller 2004 <sup>E</sup> ; Muñoz and Cavieres 2008 <sup>E</sup> ; Lázaro and Totland 2010 <sup>E</sup> ; Liao et al. 2011 <sup>E</sup> ; Yang et al. 2013 <sup>E</sup> ; Seifan et al. 2014 <sup>E</sup>

(Table 4.1 continues)

Table 4.1 (continued)

<b>Community property</b>	<b>Conditions</b>	<b>Effects on pollinator visitation</b>	<b>Effects on selection</b>	<b>References</b>
Hetero-specific plant density	Interspecific competition	Higher visitation to focal species at low heterospecific densities	Stronger selection at low densities	Thomson 1982 <sup>E</sup> ; Feinsinger 1987 <sup>T</sup> ; Feinsinger et al. 1991 <sup>E</sup> ; Lázaro and Totland 2010 <sup>E</sup> ; Vesprini and Pacini Ettore 2010 <sup>E</sup>
			No effect	Feinsinger et al. 1986 <sup>E</sup> ; Caruso 1999 <sup>E</sup> , 2002 <sup>E</sup> ; Sieber et al. 2011 <sup>E</sup> ; Wirth et al. 2011 <sup>E</sup>
Effects of co-flowering species	Facilitation or relaxed competition	Increased visitation	Weaker selection with more species	Thomson 1978 <sup>E</sup> ; Lavery 1992 <sup>E</sup> ; Kunin 1997 <sup>R</sup> ; Johnson et al. 2003 <sup>E</sup> ; Moeller 2004 <sup>E</sup> ; <b>Moeller and Geber 2005<sup>E</sup></b> ; <b>Lau et al. 2010<sup>E</sup></b> ; <b>Wassink and Caruso 2013<sup>E</sup></b> ; TerHorst et al. 2015 <sup>T</sup>
	Conflicting indirect/higher-order interactions	Visitation from pollinators with different preferences		
	Increased competition and pollen limitation	Decreased visitation	Stronger selection with more species	Waser 1986 <sup>E,T</sup> ; Stout et al. 1998 <sup>E</sup> ; <b>Caruso 2000<sup>E</sup></b> , <b>2001<sup>E</sup></b> ; Bell et al. 2005 <sup>E</sup> ; Gegear and Lavery 2005 <sup>E</sup> ; Vamosi et al. 2006 <sup>R</sup> ; Hersch and Roy 2007 <sup>E</sup> ; <b>Smith and Rausher 2008<sup>E</sup></b> ; Flanagan et al. 2010 <sup>E</sup> , 2011 <sup>E</sup> ; Arceo-Gómez and Ashman 2014 <sup>E</sup> ; TerHorst et al. 2015 <sup>T</sup> ; <b>Kooyers et al. 2017<sup>E</sup></b>
Analogous indirect/higher-order interactions	Visitation from pollinators with similar preferences	No effect		Feldman 2008 <sup>E</sup> ; <b>Lau et al. 2010<sup>E</sup></b> ; <b>Simonsen and Stinchcombe 2010<sup>E</sup></b> ; <b>Chaney and Baucom 2014<sup>E</sup></b> ; <b>Parachnowitsch et al. 2014<sup>E</sup></b>

(Cardinale et al. 2007; Benkman 2013) (Table 4.1). Alternatively, selection may be stronger if there is competition that increases variance in reproductive success and thus increases the opportunity for selection (Vamosi et al. 2006; Benkman 2013) (Table 4.1). Because facilitation or competition between co-occurring plants could be driven by pollinator sharing or by interactions with the abiotic environment, experimental tests are needed to confirm that variation in selection is pollinator-mediated (Wade and Kalisz 1990).

Of the small number of published studies that have investigated how community context may affect selection on floral traits (bolded entries in Table 4.1), most have manipulated the density of plants or presence of competitors in field or greenhouse experiments (but see Caruso 2000, 2001, 2002; Weber and Kolb 2013). As a result, there are three key gaps in our understanding of how natural variation in community context may affect selection on floral traits. First, while the density and species richness of a community jointly determine community context (Lazaro et al. 2009; Lau et al. 2010; Dietzsch et al. 2011; Essenberg 2012; but see Feldman 2008), most studies have not investigated the effects of variation in multiple aspects of community context simultaneously (see Table 4.1). Second, despite accumulating evidence that species interactions in more complex communities cannot be predicted from pairwise interactions (Strauss and Irwin 2004; Walsh 2013; Mayfield and Stouffer 2017; Eisen and Geber 2018; TerHorst et al. 2018), most examinations of the effects of co-flowering species on selection have estimated selection on a focal species in the presence or absence of one additional species ('effects of co-flowering species' studies in Table 4.1; but see Arceo-Gómez and Ashman 2014; Parachnowitsch et al. 2014). Third, most studies have estimated phenotypic selection on the floral traits of only one focal species in a community (Caruso et al. 2019). Estimating selection on more focal species within the same communities can indicate how variation in traits or evolutionary history may

contribute to how species respond to the same set of community conditions. Because species' traits may not be adaptations to the contemporary environment (e.g. Aigner 2006), these comparisons will help to determine whether different phenotypes [e.g. rewarding and deceptive orchids (Trunschke et al. 2017); early versus late phenology (Giménez-Benavides et al. 2010)] experience different patterns of selection in the same environment. Given that even closely related species often differ in ecological strategies in some ways, we expect co-occurring species to experience different magnitudes or directions of selection in the same communities, such that similarities in patterns of selection would provide evidence for a strong effect of community context.

Species in the genus *Clarkia* (Onagraceae) provide an ideal system for investigating how community context affects net and pollinator-mediated selection on floral traits. *Clarkia* generally share solitary bee pollinators that specialize on the genus (MacSwain et al. 1973), and the composition and richness of communities are generally stable over time (Lewis 1953; authors pers. obs.). These dynamics can create an opportunity for species interactions to affect the selective environment (Thompson 2005). In the southern foothills of the Sierra Nevada (Kern County, CA), four outcrossing species frequently co-occur; communities that contain a single species of *Clarkia* are equally as common as communities that contain multiple species (Eisen and Geber 2018). This natural variation in the congeneric species richness of communities creates the opportunity to estimate selection on four different species in a range of community contexts. Because *Clarkia* are the latest species to flower in this region (Lewis and Lewis 1955; MacSwain et al. 1973; Moeller 2004), we expect that intraspecific interactions or interspecific interactions with congeners have significant effects on the selective environment. Previous research in the system has generated predictions as to how community context factors may affect

patterns of selection. *Clarkia xantiana* experiences facilitation due to joint pollinator attraction (Moeller 2004), which may have led to weaker selection on floral traits in experimental populations that contained congeners (Moeller and Geber 2005). Additionally, *C. xantiana* has higher tolerance of heterospecific pollen transfer in these communities (Arceo-Gómez et al. 2016). Together, these results suggested that selection may be weaker in more species-rich *Clarkia* communities. In addition, we predicted that selection may be stronger at low conspecific or high heterospecific floral densities, given that pollen deposition is positively correlated with conspecific floral density in *C. xantiana* populations (Moeller 2004) and that high heterospecific densities can generate competition for pollination (e.g. Seifan et al. 2014). Given that pollinator visitation to and pollen limitation of *C. xantiana* varies across communities (Moeller 2004, 2005), the strength of pollinator-mediated selection is also likely to vary across communities.

In this study, we investigated how the congeneric species richness of a community and the floral density of a local neighborhood affect patterns of pollinator-mediated and net selection on floral traits of four species of *Clarkia*. Specifically, we conducted an observational study of selection in 2015 and an experimental study of pollinator-mediated selection in 2017 to test four questions. To determine if net selection on floral traits is weaker in communities with more congeneric species (**Q1**), we measured selection on four *Clarkia* species in 2015 and two *Clarkia* species in 2017 in six to ten communities (per species and year) that differed in *Clarkia* species richness. To assess whether net selection is stronger for plants surrounded by low conspecific or high heterospecific floral density (**Q2**), we analyzed how relationships between traits and fitness changed with local density in the communities where selection was estimated in 2015 and 2017. To determine if pollinator-mediated selection on floral traits varies across the above-described communities (**Q3**), we conducted a supplemental pollination experiment on two species of

*Clarkia* in 2017. Lastly, we compared all results from both years of the study across the focal species to assess whether co-flowering congeneric plants experience similar patterns of selection (Q4).

## Material and Methods

*Study system.* Out of the approximately 40 *Clarkia* species that are endemic to the Western US, many species have partially overlapping ranges, such that multiple species often co-occur, with communities containing up to six *Clarkia* species (Lewis 1953). Co-occurring species generally share pollinators, which are primarily solitary bees that specialize on the genus (MacSwain et al. 1973), but vary in floral traits including flowering time (Lewis 1961; Jonas and Geber 1999; Moeller 2004; Singh 2014), floral orientation (Lewis 1961), petal coloration (Lewis and Lewis 1955), flower size (Eisen and Geber 2018), and floral scent (Miller et al. 2014).

In the Southern Sierra Nevada (Kern River Canyon, Kern County, CA), communities contain up to four outcrossing *Clarkia* species: *C. unguiculata* Lindley, *C. cylindrica* ssp. *clavicarpa* W. Davis, *C. xantiana* ssp. *xantiana* A. Gray, and *C. speciosa* ssp. *polyantha* Harlan Lewis and M. Lewis. Hereafter we refer to each species without its subspecies designation (e.g. *C. xantiana* for *C. x. xantiana*). The species provide pollen and nectar rewards for bee visitors, and are not known to hybridize in the field (MacSwain et al. 1973). These species are all primarily outcrossing because flowers are protandrous and herkogamous: eight anthers mature over 2-3 days before the stigma becomes receptive (Lewis 1953). As such, any selfing would need to be mediated by pollinators, and outcrossing rates in *C. unguiculata* range from 0.79 to 1.0 (Vasek 1965; Ivey et al. 2016), and from 0.59 to 0.85 in *C. xantiana* (Moeller et al. 2012; Ivey et al. 2016). The species vary in flowering period and floral orientation: *C. cylindrica* (bowl-shaped flower) and *C. unguiculata* (open-faced flower) flower in early-mid May, while *C.*

*speciosa* (bowl-shaped flower) and *C. xantiana* (open-faced flower) flower in early-mid June (Moeller 2004; Singh 2014). Previous work has confirmed that *Clarkia* in the Kern River Canyon share pollinators (MacSwain et al. 1973; Moeller 2006; Singh 2014), which include generalist (polylectic) bees and ten pollen specialists (oligolectic bees) that represent four bee families (Andrenidae, Apidae, Megachilidae, and Melittidae; MacSwain et al. 1973; Moeller 2005).

*Study Overview.* To determine if net selection is weaker in communities with more congeneric species (Q1), we estimated selection on three floral traits (anther-stigma separation, floral diameter, and flower number) of all four species of *Clarkia* in 2015 and of two species of *Clarkia* in 2017. For each species, we estimated selection at six to ten communities in each year that contained between one and four species of *Clarkia* (see Table D.1 and Table D.2). To determine if net selection is stronger on plants surrounded by low conspecific or high heterospecific density (Q2), we analyzed how relationships between traits and fitness changed with local density in the communities where selection was estimated in 2015 and 2017. To determine if selection was pollinator-mediated (Q3), we conducted a supplemental pollination experiment on two species of *Clarkia* in 2017. For each of the two focal species, we estimated selection on the same three traits on open-pollinated control fruits and supplemental-pollinated fruits at nine or ten communities; the communities contained one, two, or four species of *Clarkia*. Weather during the 2015 growing season was very dry; locations throughout the range of *C. xantiana* accumulated between 160 and 200 mm of rainfall from November to June (unpublished data). Weather during the 2017 growing season was two to three times wetter than 2015, with locations throughout the range of *C. xantiana* accumulating between 400 and 600 mm of rainfall from November to June (unpublished data).

## *Field Methods*

*Q1.* To investigate how the congeneric species richness of a community affects selection, we estimated selection in all four *Clarkia* species in 2015 at 18 communities that contain one to four *Clarkia* species; there were two or three replicate communities of each community type (Table D.1). In 2017 we estimated selection in two *Clarkia* species at 16 communities that contain one, two, or four *Clarkia* species; there were three or four replicate communities of each community type (Table D.2). When possible, we worked at the same communities studied in 2015 (Table D.1; Table D.2). We defined the boundary of a community as the point at which *Clarkia* density became scarce (fewer than five plants per square meter). While some of our communities are relatively close to each other (e.g. less than 1 km apart), we consider these locations to be distinct communities because *Clarkia* are primarily pollinated by ground-nesting solitary bees that are central place foragers (MacSwain et al. 1973; Moeller 2005). The exact foraging distances of these bees are unknown, but foraging appears to occur primarily within the immediate vicinity of the nest, which matches short-distance foraging patterns that have been documented for other solitary bee species (Zurbuchen et al. 2010). In 2015 we did not include *C. unguiculata* alone communities because peak flowering at these communities occurred prior to the beginning of the study period. Across both years we chose to work at two-species communities that contain *C. cylindrica* and *C. unguiculata* and *C. speciosa* and *C. xantiana* because these pairs of species flower concurrently but have different floral orientations.

At each community, we haphazardly selected 50 focal plants per species in 2015 and between 60 and 80 focal plants per species in 2017. Focal plants were 2m apart at minimum. Two floral traits were measured in situ using calipers: anther-stigma separation and floral diameter. Anther-stigma separation can affect pollination efficiency and the degree of

outcrossing by determining where pollen is placed on pollinators and how pollinators make contact with a flower's reproductive organs (Armbruster et al. 1994; Mazer et al. 2016). If higher plant densities or the presence of co-flowering species in a community increase competition for pollination, a species may adapt by evolving less anther-stigma separation, which could lead to greater reproductive assurance via selfing (Levin 1972; Moeller and Geber 2005). Flower size traits (Strauss et al. 1996; Parachnowitsch and Kessler 2010) can increase pollinator attraction, and can also affect pollination efficiency (Galen and Newport 1987). In addition, we counted the number of open flowers.

To estimate fitness, we returned to our focal plants when the plants had set fruit, approximately three weeks after measuring plant and floral traits. We collected 1-2 fruits per focal plant; these fruits were collected from the same flowers on which we measured floral traits, or from adjacent flowers if the measured fruits had been damaged by herbivores. Fruits were only collected when the length of the fruit was intact. Fruits were stored in separate coin envelopes that were given unique ID numbers cross-referenced to the focal plant. We then counted the number of mature seeds in each fruit.

*Q2.* In 2015 and in 2017, we recorded the number of open flowers of any *Clarkia* sp. within a 1m radius of the focal individual at the time of trait measurement. We assessed density on a local scale because pollen deposition to individual flowers of *C. xantiana* increased with the density of local (within a 1m radius) conspecific neighbors (Moeller 2004), which suggests that interactions at a fine spatial scale can have a significant effect on reproductive success in these communities.

*Q3.* To determine if selection is pollinator-mediated, we manipulated pollen limitation and compared patterns of selection on fruits of supplementally-pollinated flowers to selection on fruits of open-pollinated control flowers (see methods for Q1) for two species of *Clarkia* (*C.*

*cylindrica* and *C. xantiana*) in 2017. The three pollination-traits (anther-stigma separation, floral diameter, and flower number) were measured as in 2015. On each of the 60 – 80 focal plants per species per community, we selected two female-phase flowers. Although natural selection operates on the level of the individual plant, not on the level of the individual flower, supplemental and control pollination treatments were applied to different flowers on the same plant to control for any plant-level effects on reproductive success. In addition, previous work in *Clarkia* indicates that estimates of pollen limitation from partial plant manipulations are not significantly different from estimates of pollen limitation for whole plant manipulations (Runquist and Moeller 2013), such that resource re-allocation is unlikely to bias our results. One flower was marked with a dark green sticker on the plant's stem just below the flower but not manipulated (control), while the other flower was marked with a lighter green sticker and also received a supplemental pollination treatment. The position of the control and supplemental flowers on the plant were recorded in the event that stickers did not remain on the plant. Supplemental pollen was collected from conspecific individuals >5m away from the focal plant and was applied using a toothpick and/or by brushing the anthers of a flower across the stigma. One person performed all supplemental pollinations to control for any possible variation in the quantity of pollen applied. When fruits were collected (see Q1 methods above), supplemental and control fruits were stored in separate coin envelopes that were given unique ID numbers cross-referenced to the focal plant. The number of focal plants per species per community with undamaged supplemental and control fruits ranged from 39 to 65 (average: 55 plants; Table D.2).

We conducted pollinator observations within two days of measuring the majority of focal plants at a community. Pollinator observations were conducted in four 1m<sup>2</sup> circular plots at each community. Each plot contained one focal individual and the locations of the four plots were

selected to represent the range of floral densities present at the community. One observer recorded the number of visits to the open flowers of the focal individual during a 15-minute observation period in the morning (0830-1130) and in the afternoon (1200-1500). The number of open flowers on the focal individual and on all other *Clarkia* individuals within the 1 m<sup>2</sup> plot at the time of observation were recorded. This yielded a total of four observation periods conducted in the morning and four observation periods conducted in the afternoon at each community.

*Statistical Analysis.* Most analyses (see exceptions below) were performed using linear mixed effects in R (lme4 package; Bates et al. 2015), and separate analyses were conducted on each *Clarkia* species. Models were assessed to ensure normally distributed residuals with homogenous variance. The fixed effects and dependent variables analyzed to address each question are described below. Because selection acts at the level of the population, we fit models that allowed for variation in the slopes of the relationship between fitness and traits among the replicate populations from a given community type. However, these models did not represent a significant improvement over models that allow for variation in the intercepts only among replicate communities, due to very few differences in the direction of selection across communities and similar ranges in trait and fitness values both across and within communities. As such we present the simpler (variation in intercepts only) models, where community nested within community type was included as a random effect in all models. We also present estimates of selection within each community (Table D.3) and summary statistics on the raw trait and fitness values for each community (Table D.4).

Trait values were standardized by subtracting the species mean from each individual trait value, and dividing this difference by the species standard deviation. Fitness values (the average number of seeds per fruit) were standardized by dividing individual values by the species mean.

We assessed the significance of fixed effects in these models by using the anova function in the lmerTest package ver. 2.0-29 (Kuznetsova et al. 2015) to perform type III  $F$  tests using the Satterthwaite approximation for the denominator degrees of freedom.

*Q1.* To determine if net selection on a trait varied across community types, we used an ANCOVA approach. This type of approach has been used to compare selection across populations (e.g. Caruso 2000, 2002), and here we adapt it to compare patterns of selection across community types because replicate communities generally exhibited similar patterns of selection (see *Statistical Analysis* above). We subset the entire dataset by species, and we standardized trait values and fitness values (described above) by species. Relative fitness was modeled as a function of three fixed effects: a single plant or floral trait (anther-stigma separation, floral diameter, or flower number), community type (the number of species at a community), and the interaction between the trait and community type. To calculate observed power values for each  $F$  test, we used the powerSim function in the simr package (Green and MacLeod 2016). We used the emmeans function in the emmeans package in R (Lenth 2019) to estimate net selection for each trait at each community type, and we used nonparametric bootstrapping to estimate 95% confidence intervals on the estimates of net selection (1000 iterations, boot function; Canty and Ripley 2017). We used the bias-corrected and accelerated (BCa) method, which adjusts for bias and skewness in the bootstrap distribution (Efron 1987). In general, the three traits measured were not highly correlated (Table D.5).

*Q2.* To determine if conspecific and heterospecific floral densities affected patterns of selection, relative fitness was modeled as a function of three fixed effects: a single plant or floral trait (anther-stigma separation, floral diameter, or flower number), a density measure, and the interaction between the trait and the density measure. The effects of two density measures

(absolute conspecific floral density and absolute heterospecific floral density) were tested in two separate sets of models. Models including absolute conspecific density were run using the complete dataset, while models including absolute heterospecific density were run using the subset of focal plants that were found at multi-species communities.

When the interaction between the trait and the density metric was significant, we conducted a simple slopes analysis using the `sim_slopes` function from the `jtools` package in R (Long 2018). This analysis can indicate what dynamics are driving a significant trait x density interaction by testing whether the slope of the trait-fitness relationship is different from zero at low, average, and high floral densities. These analyses were visualized using the `interact_plot` function from the `jtools` package.

*Q3.* To determine if pollinator visitation differed across community types, we used a Tweedie generalized linear mixed effects model with a Poisson power variance function and a log-link power variance function. This model was fit using the `glmer` function in the `lme4` package in R (Bates et al. 2015) and the tweedie model family specification from the `statmod` package in R (Giner and Smyth 2016). This type of model was utilized because our dataset was zero-inflated, and the response variable of interest was visitation rate, which could not be modeled as a count. The response of the average number of visits per flower per 15 minutes was modeled as a function of community type (fixed effect) and plot ID nested within community (random effects). We used the `emmeans` function in the `emmeans` package to estimate the mean pollinator visitation rate for each community type; model estimated means and 95 % confidence interval limits were back-transformed into the original units by exponentiating the output from `emmeans`. Additionally, we used a pairwise contrast in `emmeans` to determine if visitation differed across the community types.

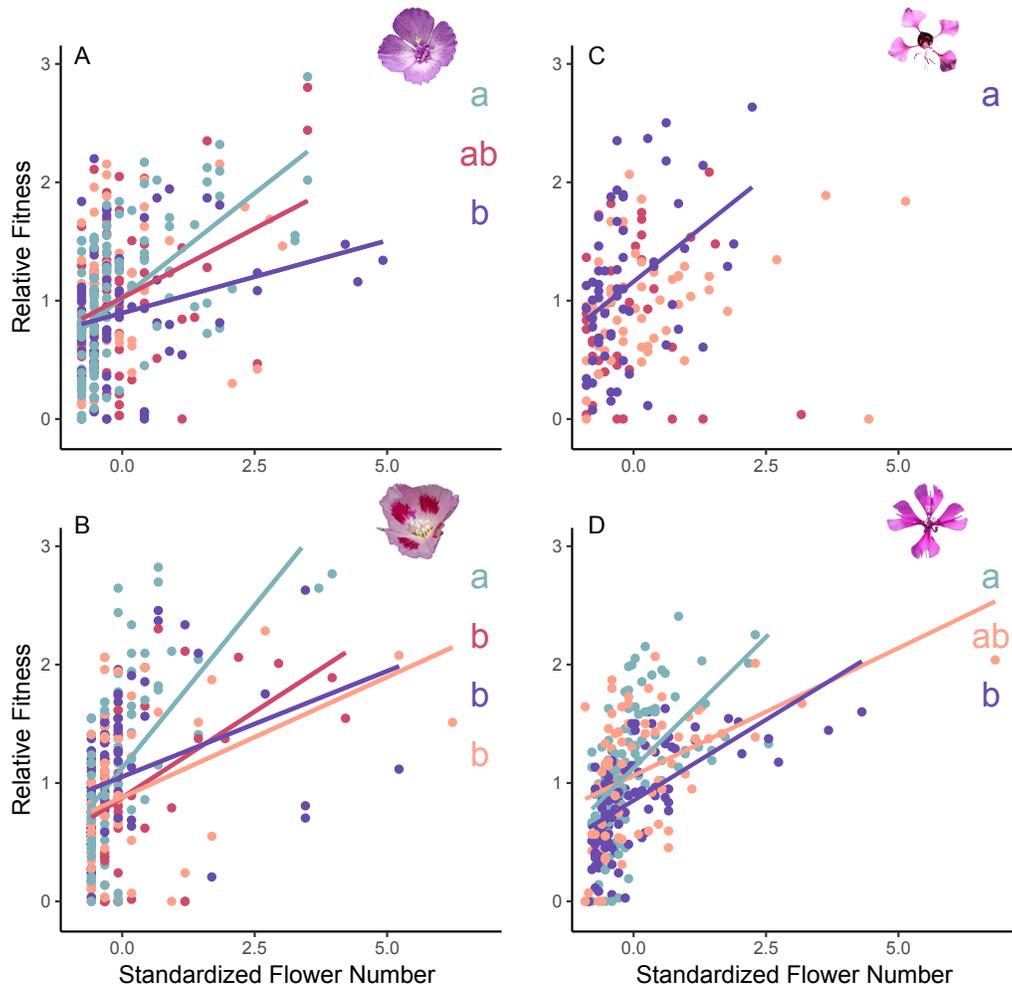
To determine if there were trait (anther-stigma separation, floral diameter, or flower number) or fitness (seed set per fruit) differences between the hand-pollination and the open-pollination treatments, we used paired t tests. A significant positive difference in seed set indicates that the hand-pollination fruits set more seeds than the open-pollination fruits, which provides evidence for pollen limitation. A significant negative difference in seed set indicates that the open-pollination fruits set more seeds than the hand-pollinated fruits, which could occur if the hand-pollination process was detrimental to seed set.

To determine if selection was pollinator-mediated in 2017, we used an ANCOVA approach (Sandring and Ågren 2009; Sletvold and Ågren 2010). Trait and fitness values were standardized by species and by community type (see above). Relative fitness was modeled as a function of three fixed effects: one of our three pollination-related traits (anther-stigma separation, floral diameter, or flower number), treatment (hand- or open-pollination) and their interaction. A significant interaction would indicate a difference in selection on the trait between the two pollination treatments. Stronger selection in the open-pollination treatment relative to the hand-pollination treatment indicates pollinator-mediated selection (Sandring and Ågren 2009; Sletvold and Ågren 2010).

## **Results**

*Q1: Is net selection weaker in communities with more species?*

In 2015, there was a significant interaction between community type and flower number in predicting relative fitness for all four species (Figure 4.1; Table D.6; Table D.7). For each species, selection was strongest in the least diverse communities (two-species communities for *C. unguiculata*, one-species communities for all other species). In other words, the number of flowers per plant had a stronger positive relationship with seed set per fruit in less diverse



**Community Type:** — One sp. — Two sp. — Three sp. — Four sp.

**Figure 4.1.** Net selection ( $S$ ) on flower number varies across community types for each species in 2015 (A: *C. cylindrica*, B: *C. speciosa*, C: *C. unguiculata*, and D: *C. xantiana*). Colors correspond to the number of species present at a given community (community type): one-species communities (blue grey), two-species communities (purple), three-species communities (raspberry), and four-species communities (coral). Trend lines are drawn through colored points when the slope at that community type was significantly different from zero. Within each species, slopes that are significantly across community types are labeled with different letters.

communities.

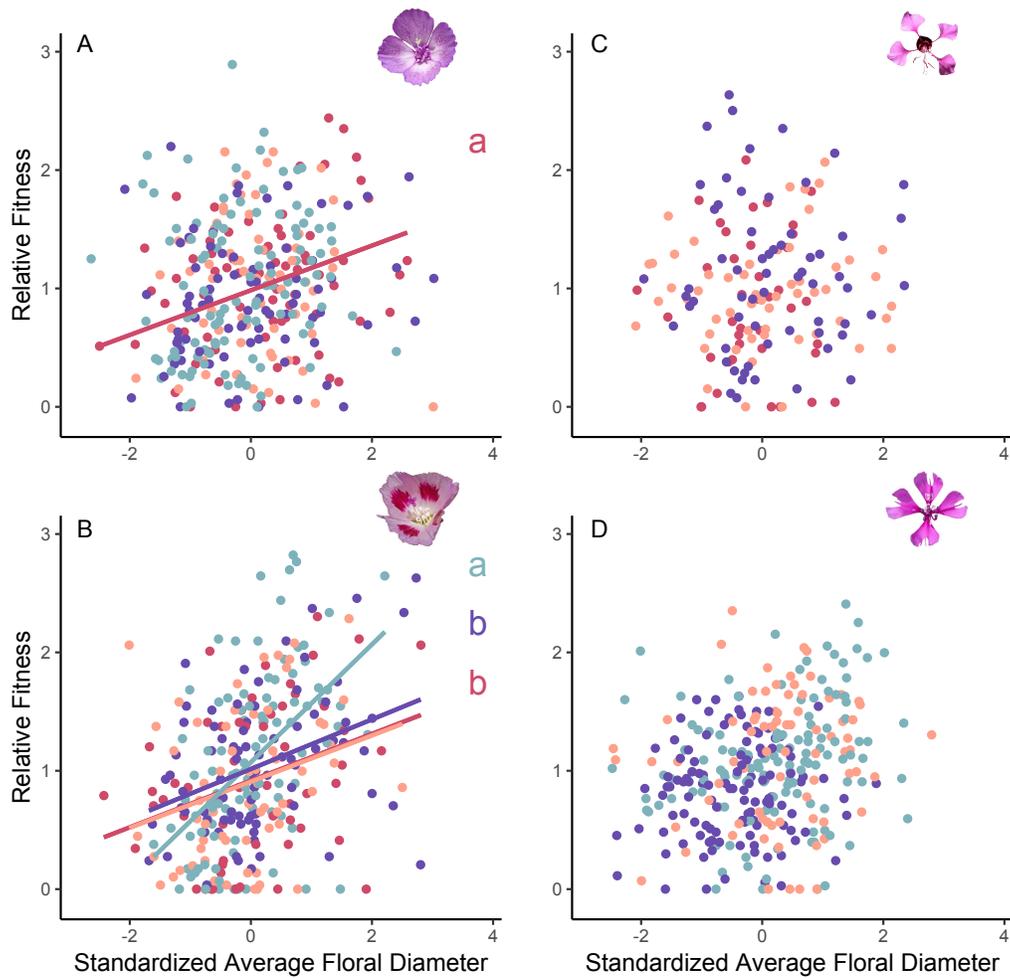
Net selection on floral diameter in 2015 varied across community types in two species (Figure 4.2; Table D.6; Table D.7). In *C. speciosa*, net selection was strongest at one-species communities compared to all other community types (Figure 4.2 B; Table D.7), while net selection on floral diameter of *C. cylindrica* was stronger at three species communities than it was at four-species communities, where it was nonsignificant (Figure 4.2 A; Table D.7). Net selection on floral diameter for *C. unguiculata* (Figure 4.2 C; Table D.7) or *C. xantiana* (Figure 4.2 D; Table D.7) did not differ across community types; there were no differences in net selection on anther stigma separation across community types in any of the four species (Table D.6; Table D.7), although these inferences may be limited by low power (Table D.6; Table D.7).

In 2017, net selection on anther-stigma separation varied across community types in *C. cylindrica* (Table D.6; Table D.8). Net selection was stronger at two-species communities than at one-species communities or four-species communities (Table D.8). There were no differences in net selection on anther stigma separation across community types for *C. xantiana* (Table D.8). Net selection on floral diameter and on flower number did not differ across community types for either species in 2017 (Table D.6; Table D.8).

*Q2: Is net selection stronger for plants surrounded by low conspecific or high heterospecific floral densities?*

Across all community types and for all species, average conspecific and heterospecific floral densities were less than ten flowers per m<sup>2</sup> in 2015 (Table D.9). In contrast, densities were generally higher in 2017: conspecific floral densities ranged from 11 to 200 flowers per m<sup>2</sup> and heterospecific floral densities ranged from 2 to 68 flowers per m<sup>2</sup> (Table D.9).

Conspecific floral density affected patterns of positive directional net selection of four



**Community Type:** — One sp. — Two sp. — Three sp. — Four sp.

Figure 4.2. Net selection (S) on average floral diameter varies across community types for some species in 2015 (A: *C. cylindrica*, B: *C. speciosa*, C: *C. unguiculata*, and D: *C. xantiana*). Colors correspond to the number of species present at a given community (community type): one-species communities (blue grey), two-species communities (purple), three-species communities (raspberry), and four-species communities (coral). Trend lines are drawn through colored points when the slope at that community type was significantly different from zero. Within each species, slopes that are significantly across community types are labeled with different letters.

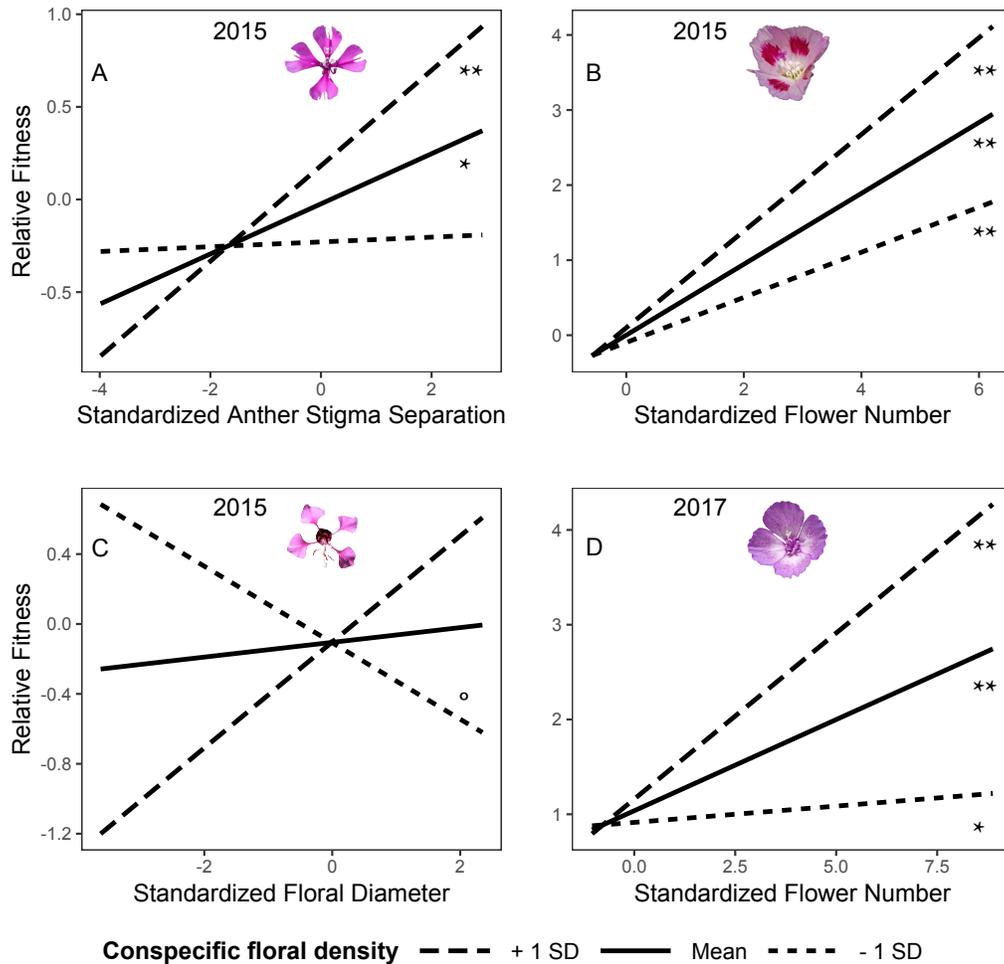
traits across all of the species and both years of the study (Figure 4.3; Table D.10; Table D.11). There was stronger net selection at higher conspecific floral densities on anther stigma separation in *C. xantiana* in 2015 (Figure 4.3 A), on flower number in *C. speciosa* in 2015 (Figure 4.3 B), and on flower number of *C. cylindrica* in 2017 (Figure 4.3 D). Floral diameter of *C. unguiculata* in 2015 displayed a similar pattern (Figure 4.3 C), with marginally significant negative directional net selection at low floral densities, compared to positive but nonsignificant directional net selection at average and higher floral densities.

Net selection varied with heterospecific floral density on two traits in 2015 and on one trait in 2017 (Figure 4.4; Table D.10; Table D.11). Net selection was strongest at low floral densities, weaker at average floral densities, and insignificant or marginally significant at high floral densities on floral diameter of *C. cylindrica* in 2015 (Figure 4.4 A) and in 2017 (Figure 4.4 C), and on flower number of *C. unguiculata* in 2015 (Figure 4.4 B).

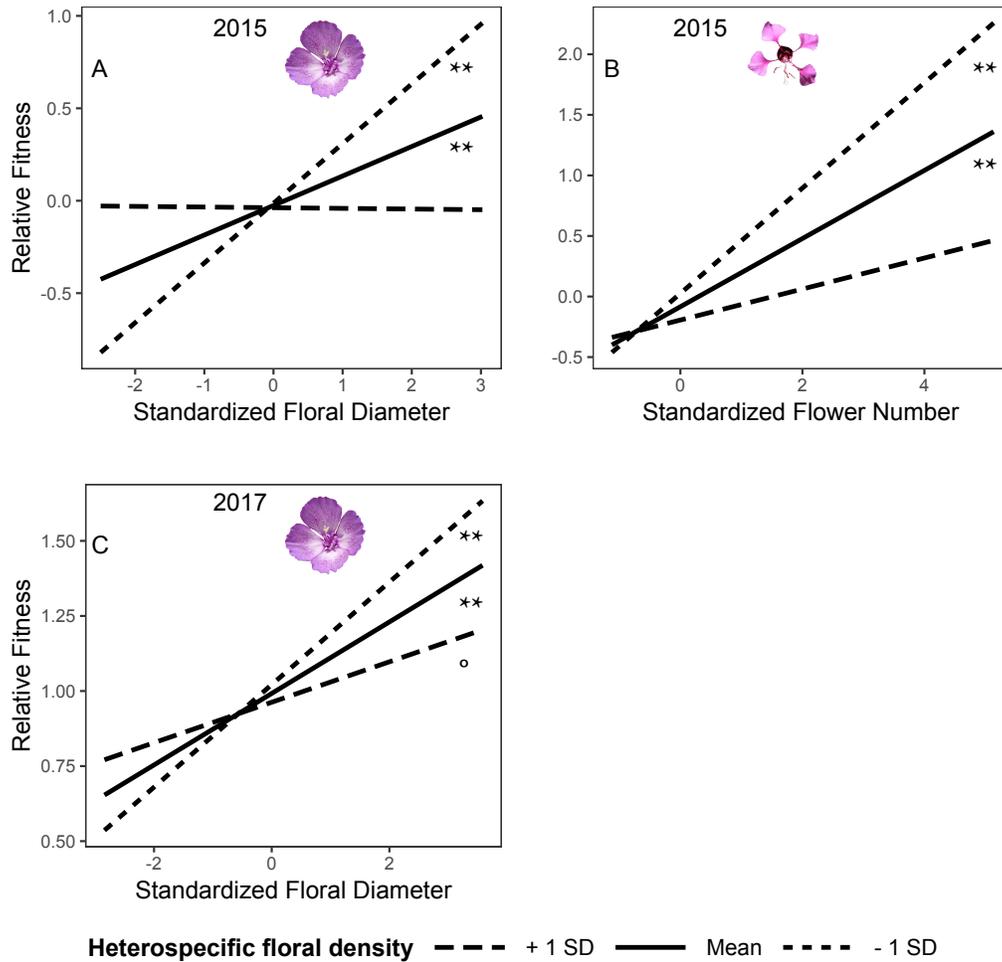
*Q3: Do pollinators impose measurable selection on floral traits in the above-described communities?*

Floral traits (anther-stigma separation and floral diameter) were measured at nine communities for *C. cylindrica* and at ten communities for *C. xantiana*. In general, trait values did not differ between the hand- and open-pollination treatment groups at each community (Table D.12).

Pollinator visitation to *C. cylindrica* varied across community types, with lower visitation (average visitation per flower per 15 minutes: 0.011) at two-species communities relative to one- and four-species communities, where the average visitation per flower per 15 minutes was 0.17 visits (Table D.13). Visitation to *C. xantiana* did not vary across the community types and ranged from 0.178 to 0.354 visits per flower per 15 minutes.



**Figure 4.3.** Visualizations of simple slope analysis for traits where conspecific floral density had a significant effect on patterns of selection for a given species in a given year (a significant density  $\times$  trait interaction; exact results of simple slope analysis in Table S6). Selection was generally stronger at high conspecific densities for anther stigma separation of *C. xantiana* in 2015 (A), flower number of *C. speciosa* in 2015 (B), floral diameter of *C. unguiculata* in 2015 (C), and flower number of *C. cylindrica* in 2017 (D). The relationship between the trait of interest and relative fitness is plotted at three conspecific floral densities: mean density + 1 SD (dashed line), mean density (solid line), and mean density – 1 SD (dotted line). Significance levels of the slopes of the lines: °:  $P < 0.1$ , \*:  $P < 0.05$ , \*\*:  $P < 0.01$ .



**Figure 4.4. Visualizations of simple slope analysis for traits where heterospecific floral density had a significant effect on patterns of selection for a given species in a given year (a significant density x trait interaction) (exact results of simple slope analysis in Table S6). Selection was generally stronger at low heterospecific densities for floral diameter of *C. cylindrica* in 2015 (A) and in 2017 (C), and for flower number of *C. unguiculata* in 2017 (B). The relationship between the trait of interest and relative fitness is plotted at three heterospecific floral densities: mean density + 1 SD (dashed line), mean density (solid line), and mean density – 1 SD (dotted line). Significance levels of the slopes of the lines: °:  $P < 0.1$ , \*:  $P < 0.05$ , \*\*:  $P < 0.01$ .**

Comparisons of seed set between the hand- and open-pollinated flowers were used to determine the extent of pollen limitation (Table D.12). Flowers in the hand-pollination treatment set more seeds than the open-pollinated control flowers at four out of the nine *C. cylindrica* communities. Of these four communities that were pollen limited, two were two-species communities and two were four-species communities (Table D.12); supplemental pollination increased seed set by 15-29 percent at these communities. At all ten *C. xantiana* communities, flowers in the hand-pollination treatment did not set more seeds than flowers in the open-pollination treatment (Table D.12).

While a few traits at a number of different community types were under selection in 2017 (Table D.8), there was only a difference in selection between the two treatments for anther-stigma separation at *C. xantiana* one-species communities (Table D.8). The hand- and open-pollinated flowers were not under selection for this trait at this community type, but the estimates of selection were opposite in sign, such that the difference between them was significant. Other traits that were under selection in the open-pollinated control group were under similar patterns of selection in the hand-pollination treatment group (Table D.8).

*Q4: Do co-flowering species experience similar patterns of selection?*

In addition to the pattern of stronger selection on flower number at communities with fewer species, which was observed for all species in 2015, two pairs of species had some similarities in patterns of selection across communities or floral densities. Floral density affected patterns of selection on floral diameter for the co-flowering species *C. cylindrica* and *C. unguiculata* (Table 4.2); *C. cylindrica* experienced stronger selection on floral diameter at low heterospecific floral densities, while *C. unguiculata* experienced stronger selection on floral diameter at high conspecific floral densities. The two bowl-shaped species *C. cylindrica* and *C.*

Table 4.2. A summary of the effects of community and neighborhood properties on estimates of selection on three floral traits of four *Clarkia* species in 2015 and two *Clarkia* species in 2017. A total of 18 estimates of selection were made: 12 in 2015 and six in 2017 (cells with thick borders, each of which is subdivided into a species composition and density sub-cell). Blank sub-cells indicate that species composition or density did not affect selection on that trait of that species in that year. A dark red-filled circle with a plus sign (+) indicates a positive relationship between the property and the strength of selection (e.g. stronger selection at higher densities or in communities with more species); a lighter red-filled circle with a plus sign (+) indicates a marginally significant relationship. In contrast, a blue-filled circle with a minus sign (-) indicates a negative relationship between the property and the strength of selection (e.g. weaker selection at higher densities or in communities with more species). An open circle indicates a property that had significant effects on patterns of selection but that the effects were not in a consistent direction. For density, all positive relationships (+) refer to conspecific density and all negative relationships (-) refer to heterospecific density.

Year	Species	Anther-stigma separation		Floral diameter		Flower number	
		Species richness	Density	Species richness	Density	Species richness	Density
2015	<i>C. cylindrica</i>			○	●-	●-	
	<i>C. unguiculata</i>				●+	●-	●-
	<i>C. speciosa</i>			●-		●-	●+
	<i>C. xantiana</i>		●+			●-	
2017	<i>C. cylindrica</i>	○			●-		●+
	<i>C. xantiana</i>						

*speciosa* both experienced an effect of congeneric species richness on selection on floral diameter in 2015 (Table 4.2).

## **Discussion**

Our study sought to determine whether patterns of net and pollinator-mediated selection on floral traits vary with the congeneric species richness of a community and the floral density of a local neighborhood for four co-occurring congeners. Across two years in which plant and floral density varied by roughly 200 %, we observed more effects of congeneric species richness on patterns of net selection in the low-density year, while the effects of floral density were fairly consistent in both years. These results suggest that the evolutionary consequences of species co-occurrence at the community level may depend on overall community density, while the local density surrounding a plant may have consistent effects on the selective environment regardless of overall community density. Additionally, we observed variation in patterns of selection on floral traits that appeared unrelated to variation in pollinator visitation and pollen limitation. This lack of concordance demonstrates the importance of continuing to evaluate the conditions under which we expect pollinator-mediated selection on floral traits versus selection mediated by other agents (e.g. Sletvold and Ågren 2014; Sapir 2017; Caruso et al. 2019). Lastly, we found similar patterns of selection among two pairs of species—one pair has the same flowering time and the other has the same floral orientation—which indicate that co-occurring species may be subject to similar patterns of selection. Together these results contribute to our understanding of the context dependency of natural selection in ecological communities, and the potential for non-pollinator-mediated selection to shape the evolution of floral traits.

### *The effects of congeneric species richness and floral density on patterns of selection*

In our two-year study of three traits of two to four focal species, we generated 18 distinct

estimates of net selection. One or more community context factor(s) modified the relationship between a trait and fitness in 11 of these 18 estimates of selection: floral density had an effect on four of 11 estimates, community type had an effect on four of 11 estimates, and both factors had an effect on three of 11 estimates (summarized in Table 4.2). Together with a small number of previous studies (see bolded references in Table 4.1), these results demonstrate that biotic interactions both at the local- and community-level can affect the strength of selection (e.g. Sletvold et al. 2013; Sletvold and Ågren 2014). Because few studies have tested if selection on floral traits varies with floral density (Donohue et al. 2000; Caruso 2002; Stanton et al. 2004; Weber and Kolb 2013), our ability to predict how density will affect patterns of selection from the ecological and evolutionary context of a community is currently limited. In contrast, a larger body of literature has examined whether the presence of co-flowering species affects patterns of selection (see bolded references in Table 4.1). Our results generally follow the expected pattern of weaker selection when co-occurring species are facilitative (Moeller and Geber 2005) or when competition is weak and asymmetric (Wassink and Caruso 2013). Below we discuss what types of interactions between plants and local community conditions may drive these patterns, and what additional data would be needed to attribute these patterns to specific drivers.

*The effects of conspecific and heterospecific floral density were consistent across years*

Across both years of our study, we generally observed stronger selection on floral traits at high conspecific floral densities (Figure 4.3; Table 4.2). This result was contrary to our expectation of stronger selection at low conspecific densities, which was based on the positive relationship between conspecific floral density and pollen deposition observed in *C. xantiana* (Moeller 2004) and many other systems (reviewed in Ghazoul 2005). This result was also contrary to findings from other systems where the strength of selection on a number of floral

traits did not vary with the abundance of conspecifics or competitors (Donohue et al. 2000; Caruso 2002; Stanton et al. 2004; Weber and Kolb 2013). Stronger selection at high local conspecific floral densities indicates that a change in a trait value has a larger effect on fitness when a focal plant is surrounded by more neighboring plants, which could be due to intraspecific competition for pollinators or resources at high densities. Experimentally manipulating both abiotic resources and access to pollinators would indicate if and under what circumstances the abiotic environment may be a significant agent of selection on floral traits in this system (Caruso et al. 2005; Sletvold et al. 2017).

We also observed stronger selection for larger flowers in *C. cylindrica* and for more flowers in *C. unguiculata* at low heterospecific floral densities (Figure 4.4, Table 4.2). These two species co-occur more frequently than expected by chance (Eisen and Geber 2018), which suggests these species may experience interspecific facilitation where they co-occur at low densities (Rathcke 1983; Muñoz and Cavieres 2008; Seifan et al. 2014). In general, stronger selective pressure is expected under high heterospecific densities that lead to interspecific competition for pollination (e.g. Feinsinger 1987). However, facilitation can generate selection on different traits that would promote pollinator constancy (Armbruster et al. 1994; Grant 1994; Gumbert et al. 1999; Moeller 2004; Sargent and Ackerly 2008; Waelti et al. 2008).

*The effects of congeneric species richness were more prevalent in the low-density year (2015)*

Of the seven significant interactions between the congeneric species richness of a community and selection on a trait (summarized in Table 4.2), six occurred during 2015, when density was considerably lower relative to 2017. Within this pattern of more congeneric species richness effects during the low-density year, a striking result of our study was that all four species generally experienced weaker selection on flower number in communities with more

species in 2015 (Figure 4.2). Because reduced intraspecific competition can lead to higher productivity in more diverse communities (Johnson et al. 2006; Cardinale et al. 2007, 2011), facilitation or the relaxation of competition are expected to lead to weaker patterns of selection in more species-rich communities (Moeller and Geber 2005; Wassink and Caruso 2013; Parachnowitsch et al. 2014). In our study, this pattern was not present in the year with higher density (2017), which suggests that intraspecific competition may have predominated and generated the same effects on focal species regardless of the community context (e.g. Wirth et al. 2011). Interactions between plant species that range from facilitative to competitive have been observed in a number of systems (e.g. Moragues and Traveset 2005; Muñoz and Cavieres 2008; Seifan et al. 2014) and are generally attributed to the presence of an additional species facilitating joint pollinator attraction at low densities but becoming a competitor for pollination at high densities.

#### *Pollinators as an agent of selection*

Because selection on floral traits may be driven by a number of abiotic or biotic agents of selection, we conducted an experimental test of pollinator-mediated selection in 2017. We found little evidence for pollinator-mediated selection but different patterns between pollinator visitation and pollen limitation in the two focal species. In *C. xantiana*, pollinator visitation rates did not differ across community types, there was no evidence of pollen limitation, and no differences in selection between the hand and open pollinated treatments at any community type (Tables D.8, D.12, D.13). These patterns indicate that any selection on floral traits of *C. xantiana* was likely not mediated by pollinators in 2017, which could result from high floral densities decreasing the opportunity for selection (Richards et al. 2009; Benkman 2013; Trunschke et al. 2017), or from post-pollination processes that strongly modify the signal of pollinator-mediated

selection (Totland 2004; Caruso et al. 2005). Given that floral traits of *C. xantiana* have been under selection in previous studies (Moeller and Geber 2005; Runquist et al. 2017), we suspect that the lack of pollinator-mediated selection in 2017 was the result of high mate availability and a low opportunity for selection, rather than the effects of post-pollination processes. In addition, we estimated selection exclusively via a component of female fitness, seed set, but both the direction and magnitude of selection can vary between male and female function in a number of systems (O'Connell and Johnston 1998; Sahli and Conner 2011; Kulbaba and Worley 2012), including *Clarkia* (Runquist et al. 2017).

The patterns of visitation, pollen limitation, and pollinator-mediated selection observed for *C. cylindrica* join a growing body of literature that does not support two key expectations about these dynamics. First, in contrast to the expectation that pollinator availability will determine reproductive success (Knight et al. 2005; Benkman 2013), *C. cylindrica* experienced low pollinator visitation at two-species communities and higher visitation at four-species communities, but was pollen-limited at both types of communities. Variation in pollinator visitation that does not correspond to variation in pollen limitation has also been observed in other systems, including *Crocus vernus* (Totland et al. 1998), and *Asclepias syriaca* (Caruso et al. 2005). Together with our results from *C. cylindrica*, these studies suggest that additional factors beyond pollinator visitation are likely important determinants of fitness, such as pollinator efficiency or effectiveness (Campbell et al. 1991; Eckhart et al. 2006; Koski et al. 2018), abiotic resource limitation (Campbell and Halama 1993; Ashman and Morgan 2004; Sapir 2017), and herbivory (Gómez 2003; Bartkowska and Johnston 2015). Second, while theoretical (Reynolds et al. 2009; Benkman 2013) and some empirical studies (Sletvold and Ågren 2016; Trunschke et al. 2017) have demonstrated a positive correlation between the strength of

pollinator-mediated selection and the degree of pollen limitation, our data suggest that selection was not pollinator-mediated, even in populations that were pollen-limited (no difference between the strength of selection in the hand- and open-pollinated treatments; Table D.8). Patterns of selection that do not follow from patterns of pollen limitation have occurred in other systems due to correlational selection (Campbell and Bischoff 2013) and variation in selection that was driven more by a change in the functional significance of traits than by variation in interaction intensity (Sletvold and Ågren 2014). Taken together, these results reflect the complex processes that shape patterns of selection on floral traits (reviewed in Caruso et al. 2019) and reinforce the idea that pollinator-mediated selection may be nonexistent or difficult to detect if selection is primarily mediated by other agents or if current phenotypes represent a response to past episodes of pollinator-mediated selection (e.g. Aigner 2006).

#### *Variation in patterns of selection across closely related species in a community*

Given limited previous research into variation in selection on co-occurring species, a goal of our study was to compare patterns of selection on multiple *Clarkia* species that frequently co-occur in the Kern River Canyon (Kern County, CA). Across two pairs of species, one that has the same flowering time and one that has the same floral orientation, similarities in patterns of selection reinforce our previously discussed finding of intraspecific competition at high floral densities and interspecific facilitation at low floral densities. The co-flowering species *C. cylindrica* and *C. unguiculata* both experienced an effect of floral density on selection on floral diameter—*C. unguiculata* was under stronger selection at higher conspecific floral densities, while *C. cylindrica* was under stronger selection at lower heterospecific floral densities. These patterns could result from intraspecific competition at high floral densities and interspecific facilitation at low floral densities (see above). In addition, the two bowl shaped species *C.*

*cylindrica* and *C. speciosa* both experienced an effect of floral density on selection on flower size in 2015, which also suggests that these species may experience facilitation in more species-rich communities (Parachnowitsch et al. 2014). The results of our two-year study suggest that community context had similar effects on patterns of selection on a number of co-occurring species (Irwin 2000). More studies of natural selection on multiple co-occurring congeners are needed to provide insight into the aspects of community context that tend to facilitate similarities or differences in patterns of selection.

### *Conclusions*

Our study contributes to the growing body of literature that suggests the evolution of floral traits is not only determined by pollinator-mediated selection but also by other agents of selection (Strauss and Whittall 2006; Caruso et al. 2019). Conspecific and heterospecific floral density and congeneric species richness are properties of *Clarkia* communities that affected patterns of net selection on floral traits, which could be non-pollinator-mediated. In particular, our results are consistent with facilitation in species-rich communities leading to weaker patterns of selection, which could be further tested by comparing patterns of selection in communities along a competition to facilitation gradient. While the strength of the effects of floral density and congeneric species richness varied across species and with the conditions of a community in a given year, our results suggest that in general, intraspecific competition at high floral densities and interspecific competition at low floral densities may be key species interactions among co-occurring plants that affect selection on floral traits. Because the effects of these interactions occurred in the absence of pollinator-mediated selection, we speculate that community context likely affects selection mediated by resource competition in many systems.

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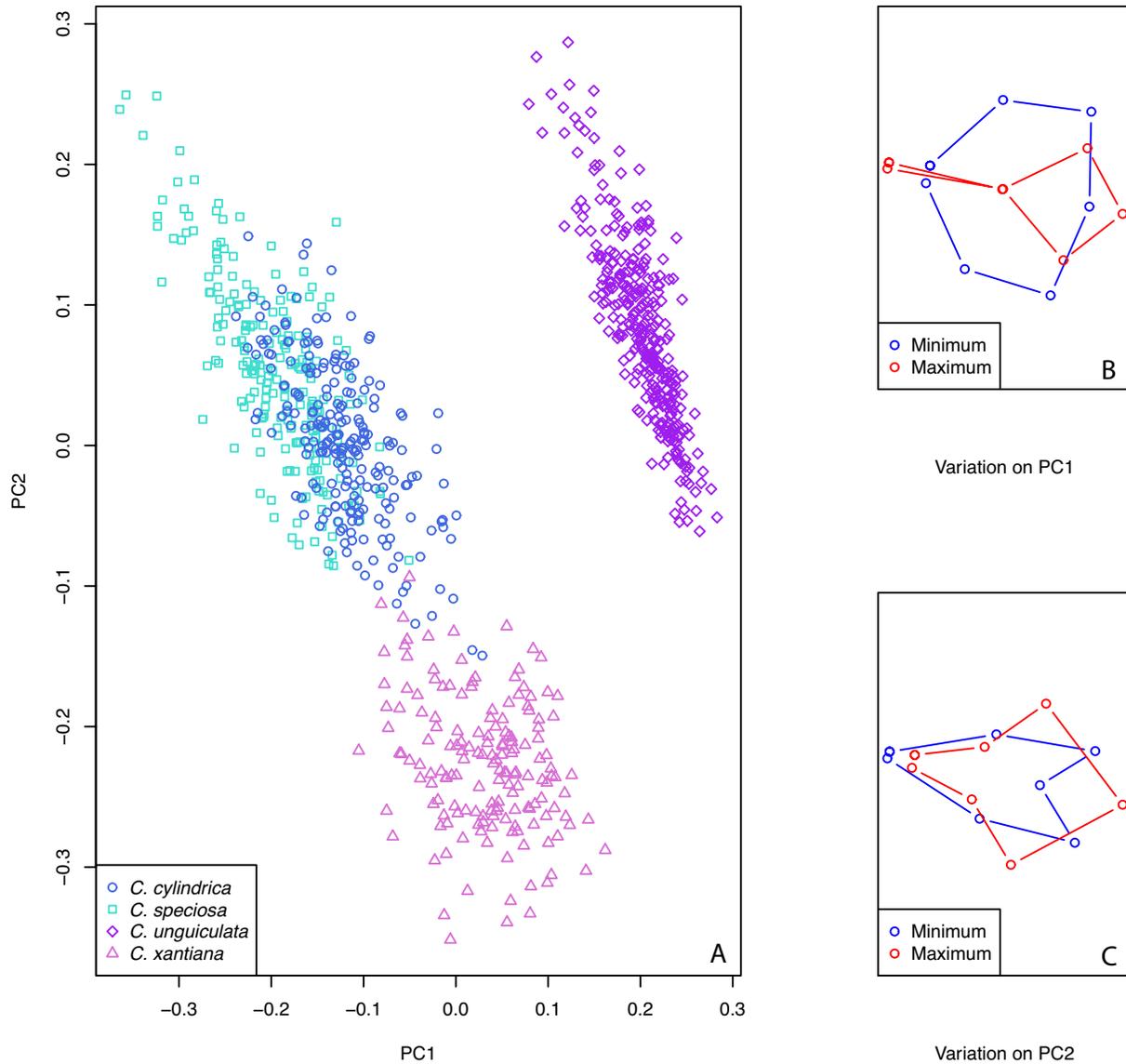
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APPENDIX A

CHAPTER 1 APPENDIX

Table A.1. The names, locations, elevations, and species compositions of each of the 14 communities used in the study, and the number of plants measured for plant and floral traits. Species *C* is *C. cylindrica*, species *S* is *C. speciosa*, species *U* is *C. unguiculata*, and species *X* is *C. xantiana*.

Com. name	Species present	Plant traits		Floral traits		Easting	Northing	Elevation (m)
		<i>N</i> families	<i>N</i> plants	<i>N</i> families	<i>N</i> plants			
SF	<i>C</i>	9	19	9	16	362072	3938241	739
45	<i>C</i>	10	25	10	21	362204	3939127	780
41	<i>S</i>	10	18	10	21	361923	3940091	739
60	<i>S</i>	8	20	8	17	363695	3939440	792
31.5	<i>U</i>	10	28	10	26	360523	3937961	764
33	<i>U</i>	10	30	10	26	340866	3926104	360
BR	<i>X</i>	8	16	7	10	362540	3939354	799
LCWW	<i>X</i>	7	14	6	10	344943	3927455	480
30	<i>C</i>	9	18	7	8	345580	3945328	887
	<i>U</i>	10	29	10	27			
34	<i>C</i>	10	27	10	20	343207	3926804	441
	<i>U</i>	10	28	9	21			
GRE	<i>S</i>	6	11	6	12	363609	3940311	817
	<i>X</i>	8	19	8	17			
S8	<i>S</i>	7	15	7	15	363018	3939611	812
	<i>X</i>	9	18	9	17			
DEM 38	<i>C</i>	10	30	9	23	349530	3932818	619
	<i>S</i>	9	21	9	18			
	<i>U</i>	10	28	10	26			
	<i>X</i>	5	10	4	8			
MCK	<i>C</i>	10	22	10	19	353371	3933462	723
	<i>S</i>	10	20	10	23			
	<i>U</i>	9	24	9	20			
	<i>X</i>	10	23	10	16			



**Figure A.1.** Individual plants' values along petal shape PC axis 1 (58.7 % of variation) and PC axis 2 (32.2 % of variation); *C. cylindrica* (blue open circles), *C. speciosa* (aqua open squares), *C. xantiana* (light pink open triangles), and *C. unguiculata* (purple open diamonds) (A). The minimum shape along PC1 (B; blue circles and lines) approximates a pentagon, while the maximum shape resembles a club (B; red circles and lines). The minimum shape along PC2 (C; blue circles and lines) resembles a heart shape, while the maximum shape approximates a diamond or kite shape (C; red circles and lines).

Table A.2. Models used in common garden data analysis. Models follow the R conventions of model writing, where “x” indicates three fixed effects will be in the model: a fixed effect for each term and the interaction between the two terms; the notation (1|Community:Family:Plant) indicates a random effect where plant is nested within family which is nested within community.

<b>Question</b>	<b>Model number</b>	<b>Type of response variable</b>	<b>Dataset</b>	<b>Type of model</b>	<b>Model</b>
Does trait variation have a heritable component?	1.1	Floral trait	Data from 2-4 female phase flowers per plant from all plants of all species	General linear mixed effects model	Trait ~ Community Type + (1 Community:Family:Plant)
	1.2	Height	Data from all plants all species	General linear mixed effects model	Trait ~ Community Type + (1 Community:Family)
	1.3	Date of first flower	Data from all plants all species	Generalized linear model fit with a Poisson distribution	Trait ~ Community Type + (1 Community:Family)
Does community type affect patterns of trait expression among correlated floral traits?	2.1	All six floral traits	Data from a pair of species; repeated for all pairs	General linear mixed effects model	Trait <sub>1</sub> + Trait <sub>1</sub> Trait <sub>1</sub> Trait <sub>1</sub> Trait <sub>1</sub> Trait <sub>1</sub> ~ Community Type x Species + (1 Community:Family)
Does community type affect patterns of trait expression?	2.2	Floral trait or height	Data from a pair of species; repeated for all pairs	General linear mixed effects model	Trait ~ Community Type x Species + (1 Community:Family)
	2.3	Date of first flower	Data from a pair of species; repeated for all pairs	Generalized linear model fit with a Poisson distribution	Trait ~ Community Type x Species + (1 Community:Family)

Table A.3. Linear mixed effects models were used to test the significance of family- and plant-level variation in each trait for each species (models are detailed in Table A.2). Values are chi-square test statistics that compared twice the difference in the log-likelihood of models with versus without the factor of interest. Sample sizes refer to the number of flowers or plants used as replicates for the floral traits or the plant traits, respectively. For some species, traits that were square root transformed (<sup>a</sup>) or cube root transformed (<sup>b</sup>). *P* values are indicated as follows: ° : *P* < 0.1; \* : *P* < 0.05; \*\* : *P* < 0.01; \*\*\* : *P* < 0.001.

Species	<i>N</i>	Trait	Family	Plant
<i>C. xantiana</i>	154	Style length	27.63***	33.94***
		Floral diameter	16.44***	45.81***
		Petal area	16.96***	83.44***
		Petal area: diameter ratio	9.97**	44.06***
		Shape PC1	20.20***	32.06***
		Shape PC2	17.19***	28.82***
	105	Height	3.78°	
		Flowering Time <sup>b</sup>	13.86***	
<i>C. speciosa</i>	200	Style length	38.46***	59.63***
		Floral diameter	10.55**	58.92***
		Petal area	33.31***	117.03***
		Petal area: diameter ratio	8.40**	32.04***
		Shape PC1	6.60*	77.55***
		Shape PC2	14.34***	39.72***
	119	Height	0.00	
		Flowering Time <sup>a</sup>	0.03	
<i>C. cylindrica</i>	214	Style length	63.75***	29.30***
		Floral diameter	9.80**	82.67***
		Petal area	28.75***	107.47***
		Petal area: diameter ratio	9.43**	47.60***
		Shape PC1	33.87***	67.76***
		Shape PC2	6.98**	32.60***
	147	Height <sup>a</sup>	5.30*	
		Flowering Time <sup>a</sup>	2.99°	
<i>C. unguiculata</i>	300	Style length	35.87***	67.27***
		Floral diameter	32.65***	117.78***
		Petal area	33.97***	151.76***
		Petal area: diameter ratio	20.16***	92.31***
		Shape PC1	25.91***	88.26***
		Shape PC2	22.50***	118.01***
	168	Height <sup>a</sup>	9.22**	
		Flowering Time <sup>a</sup>	11.16***	

Table A.4. MANOVA models (details of the model in Table A.2) of floral traits for each species pair.

<b>Flowering</b>	<b>Species</b>	<b>Term</b>	<b>MS</b>	<b>NDF</b>	<b>DDF</b>	<b>F</b>	<b>P</b>
Co-flowering	<i>C. cylindrica</i> & <i>C. unguiculata</i>	Type	116.64	2	3.860	3.014	0.163
		Species	244.79	1	8.369	6.327	<b>0.035</b>
		Type x Species	275.20	2	12.802	7.113	<b>0.008</b>
Co-flowering	<i>C. speciosa</i> & <i>C. xantiana</i>	Type	152.26	2	3.668	2.822	0.181
		Species	4.547	1	30.003	0.084	0.774
		Type x Species	119.55	2	27.999	2.215	0.128
Non-co-flowering	<i>C. xantiana</i> & <i>C. unguiculata</i>	Type	14.94	1	2.855	0.508	0.530
		Species	40.58	1	5.118	1.379	0.292
		Type x Species	44.61	1	5.118	1.516	0.272
Non-co-flowering	<i>C. cylindrica</i> & <i>C. speciosa</i>	Type	249.95	1	2.665	3.701	0.162
		Species	1103.04	1	6.124	16.33	<b>0.007</b>
		Type x Species	15.76	1	6.124	0.233	0.646
Non-co-flowering	<i>C. unguiculata</i> & <i>C. speciosa</i>	Type	8.08	1	2.759	0.153	0.724
		Species	15.39	1	5.529	0.291	0.611
		Type x Species	434.86	1	5.529	8.220	<b>0.031</b>
Non-co-flowering	<i>C. cylindrica</i> & <i>C. xantiana</i>	Type	103.30	1	2.224	3.141	0.206
		Species	0.35	1	7.721	0.011	0.920
		Type x Species	114.81	1	7.721	3.491	0.100

Table A.5. Tukey’s honest significant difference post-hoc tests on the differences between *C. cylindrica* and *C. unguiculata* in the given trait at each community type. Positive estimates indicate that *C. cylindrica* has larger trait values than *C. unguiculata*. General linear hypothesis tests determine the differences between the two species at two community types; for instance, the first row of the table compares the difference in floral diameter between *C. cylindrica* and *C. unguiculata* at one-species communities with the difference in floral diameter between the species at two-species communities. Positive estimates indicate the first community type in the hypothesis has a larger difference in the trait relative to the second community type in the hypothesis. All tests were corrected for multiple comparisons.

<b>Tukey’s HSD tests</b> <i>Testing differences between species at each community type</i>					<b>General linear hypothesis tests</b> <i>Testing differences of the differences</i>			
Trait	Com. Type	Estimate ± 1 SE	<i>t</i> ratio	<i>P</i>	Hypothesis	Estimate ± 1 SE	<i>Z</i> value	<i>P</i>
Floral diameter	One	- 4.53 ± 1.59	- 2.892	<b>0.032</b>	Δ One =	- 9.97 ± 1.96	- 5.08	<b>&lt; 0.001</b>
	Two	5.44 ± 1.21	4.499	<b>&lt; 0.001</b>	Δ Four =	- 6.17 ± 1.58	- 3.91	<b>&lt; 0.001</b>
	Four	- 0.73 ± 1.04	- 0.699	0.485	Δ Two =	3.80 ± 1.88	2.02	0.106
Petal area	One	0.17 ± 0.18	0.955	0.390	Δ One =	- 0.90 ± 0.20	- 4.65	<b>&lt; 0.001</b>
	Two	1.08 ± 0.08	12.793	<b>&lt; 0.001</b>	Δ Four =	- 0.42 ± 0.11	- 3.76	<b>&lt; 0.001</b>
	Four	0.66 ± 0.07	9.091	<b>&lt; 0.001</b>	Δ Two =	0.49 ± 0.19	2.58	<b>0.025</b>

Table A.6. Tukey's honest significant difference post-hoc tests on the differences between *C. unguiculata* and *C. speciosa* in the given trait at each community type. Positive estimates indicate that *C. speciosa* has larger trait values than *C. unguiculata*. General linear hypothesis tests determine the differences between the differences at a pair of community types. Positive estimates indicate the first community type in the hypothesis has a larger difference in the trait relative to the second community type in the hypothesis. All tests were corrected for multiple comparisons.

<b>Tukey's HSD tests</b> <i>Testing differences between species at each community type</i>					<b>General linear hypothesis tests</b> <i>Testing differences of the differences</i>			
Trait	Com. Type	Estimate ± 1 SE	<i>t</i> ratio	<i>P</i>	Test	Estimate ± 1 SE	Z value	<i>P</i>
Floral diameter	One	- 1.30 ± 1.78	- 0.73	0.506	Δ Four = Δ One	6.04 ± 2.21	2.73	<b>0.0063</b>
	Four	4.74 ± 1.33	3.55	<b>0.0005</b>				
Petal area	One	1.86 ± 0.12	15.58	< <b>0.0001</b>	Δ Four = Δ One	0.80 ± 0.16	4.97	< <b>0.0001</b>
	Four	2.66 ± 0.11	24.07	< <b>0.0001</b>				

Table A.7. Tukey's honest significant difference post-hoc tests on the differences between two species in date of first flower at each community type. Positive estimates indicate that the first species in the pair has larger trait values than the second species. General linear hypothesis tests on the differences between the differences at a pair of community types. Positive estimates indicate the first community type in the hypothesis has a larger difference in the trait relative to the second community type in the hypothesis.

<i>Testing differences between species</i>				<i>Testing differences of the differences</i>			
Species	Com. Type	Z ratio	P	Hypothesis	Estimate ± 1 SE	Z value	P
<i>C. cylindrica</i> – <i>C. unguiculata</i>	One	5.602	<b>&lt;0.001</b>	Δ One =	0.47 ± 0.13	3.58	<b>0.001</b>
	Two	3.870	<b>&lt;0.001</b>	Δ Two			
	Four	7.859	<b>&lt;0.001</b>	Δ Four =	0.17 ± 0.07	2.43	<b>0.038</b>
				Δ Two			
				Δ Four =	-0.30 ± 0.13	-2.29	0.054
				Δ One			
<i>C. speciosa</i> – <i>C. xantiana</i>	One	2.932	<b>0.003</b>	Δ One =	0.38 ± 0.18	2.17	0.069
	Two	2.915	<b>0.004</b>	Δ Two			
	Four	8.674	<b>&lt;0.001</b>	Δ Four =	0.31 ± 0.06	4.84	<b>&lt;0.001</b>
				Δ Two			
				Δ Four =	-0.07 ± 0.18	-0.38	0.919
				Δ One			

Table A8. Mean  $\pm$  1 SE of all traits by community type. Traits are abbreviated as: PADR—petal area to floral diameter ratio; FD—floral diameter; DAY—date of first flower; HGT—height at flowering; PA—petal area; PC1—floral shape PC1 value; PC2—floral shape PC2 value; STY—style length. Species are abbreviated as: C—*C. cylindrica*; S—*C. speciosa*; U—*C. unguiculata*; X—*C. xantiana*. The number listed in the community type column indicates the number of species present in the community.

Trait	Species	Community Type	Mean	SE
PADR	C	One	0.904	0.006
		Two	0.824	0.005
		Four	0.940	0.006
	S	One	1.842	0.167
		Two	1.740	0.139
		Four	1.598	0.092
	U	One	0.574	0.026
		Two	0.568	0.025
		Four	0.533	0.029
	X	One	0.840	0.050
		Two	0.921	0.042
		Four	0.873	0.040
FD	C	One	26.832	0.162
		Two	36.460	0.215
		Four	29.497	0.140
	S	One	30.115	1.410
		Two	30.812	1.196
		Four	35.110	1.084
	U	One	31.306	0.578
		Two	30.979	0.658
		Four	30.382	0.687
	X	One	33.265	0.983
		Two	29.405	0.692
		Four	3.588	0.888
DAY	C	One	34.956	2.646
		Two	25.277	1.949
		Four	26.745	2.058
	S	One	52.114	2.268
		Two	48.793	2.638
		Four	45.267	2.271
	U	One	18.483	1.351
		Two	22.707	1.141
		Four	18.442	1.496
	X	One	35.813	2.368
		Two	43.825	2.119
		Four	27.818	2.297

(Table A.8 continues)

Table A.8 (continued)

<b>Trait</b>	<b>Species</b>	<b>Community Type</b>	<b>Mean</b>	<b>SE</b>
HGT	C	One	55.947	2.514
		Two	49.815	2.395
		Four	49.524	2.208
	S	One	63.398	2.078
		Two	63.169	2.839
		Four	61.427	2.323
	U	One	64.464	2.632
		Two	70.843	2.177
		Four	60.565	2.359
	X	One	74.084	3.685
		Two	85.210	3.129
		Four	72.376	3.843
PA	C	One	1.250	0.070
		Two	2.154	0.120
		Four	1.562	0.067
	S	One	2.827	0.128
		Two	3.018	0.171
		Four	3.589	0.115
	U	One	1.067	0.037
		Two	1.022	0.027
		Four	0.909	0.028
	X	One	1.771	0.080
		Two	1.512	0.046
		Four	1.890	0.073
PC1	C	One	-0.127	0.001
		Two	-0.119	0.002
		Four	-0.117	0.001
	S	One	-0.192	0.009
		Two	-0.193	0.010
		Four	-0.204	0.008
	U	One	0.196	0.004
		Two	0.207	0.005
		Four	0.210	0.004
	X	One	0.023	0.011
		Two	0.027	0.007
		Four	0.050	0.011
PC2	C	One	0.001	0.001
		Two	0.005	0.002
		Four	0.004	0.001
	S	One	0.046	0.010
		Two	0.051	0.012
		Four	0.062	0.009

(Table A.8 continues)

Table A.8 (continued)

<b>Trait</b>	<b>Species</b>	<b>Community Type</b>	<b>Mean</b>	<b>SE</b>
PC2	U	One	0.102	0.009
		Two	0.067	0.009
		Four	0.068	0.008
	X	One	-0.216	0.010
		Two	-0.224	0.008
		Four	-0.236	0.007
STY	C	One	10.972	0.054
		Two	13.932	0.067
		Four	11.867	0.055
	S	One	11.669	0.347
		Two	12.192	0.421
		Four	11.719	0.453
	U	One	16.792	0.438
		Two	17.023	0.387
		Four	14.082	0.319
	X	One	14.161	0.555
		Two	13.074	0.292
		Four	13.828	0.408

Table A.9. Mean  $\pm$  1 SE of all traits by community. Traits are abbreviated as: PADR—petal area to floral diameter ratio; FD—floral diameter; DAY—date of first flower; HGT—height at flowering; PA—petal area; PC1—floral shape PC1 value; PC2—floral shape PC2 value; STY—style length. Species are abbreviated as: C—*C. cylindrica*; S—*C. speciosa*; U—*C. unguiculata*; X—*C. xantiana*. The number listed in the community type column indicates the number of species present in the community. The community abbreviations correspond with those in Table A.1.

Trait	Species	Community Type	Community	Mean	SE	
PADR	C	One	45	0.870	0.041	
		One	SF	0.950	0.058	
		Two	30	0.831	0.033	
		Two	34	0.821	0.038	
		Four	DEM 38	0.932	0.047	
		Four	MCK	0.951	0.058	
	S	One	41	1.564	0.149	
		One	60	2.185	0.311	
		Two	GRE	1.737	0.241	
		Two	S8	1.743	0.168	
		Four	DEM 38	1.414	0.107	
		Four	MCK	1.743	0.136	
	U	One	31.5	0.525	0.029	
		One	33	0.624	0.042	
		Two	30	0.550	0.033	
		Two	34	0.591	0.039	
		Four	DEM 38	0.574	0.041	
		Four	MCK	0.480	0.035	
		X	One	BR	0.972	0.070
			One	LCWW	0.708	0.042
Two	GRE		0.922	0.043		
Two	S8		0.919	0.073		
Four	DEM 38		0.877	0.064		
Four	MCK		0.870	0.052		
FD	C	One	45	28.556	1.253	
		One	SF	24.568	1.436	
		Two	30	32.686	1.043	
		Two	34	37.970	1.413	
		Four	DEM 38	30.973	1.243	
		Four	MCK	27.709	1.231	
	S	One	41	32.289	2.102	
		One	60	27.430	1.622	
		Two	GRE	29.415	2.262	
		Two	S8	31.929	1.166	
		Four	DEM 38	36.134	1.357	

(Table A.9 continues)

Table A.9 (continued)

<b>Trait</b>	<b>Species</b>	<b>Community Type</b>	<b>Community</b>	<b>Mean</b>	<b>SE</b>	
FD	S	Four	MCK	34.308	1.620	
		One	31.5	30.789	0.808	
	X	One	33	31.824	0.831	
		Two	30	30.699	0.890	
		Two	34	31.340	0.995	
		Four	DEM 38	29.790	0.940	
		Four	MCK	31.152	1.001	
		One	BR	31.060	1.328	
		One	LCWW	35.471	1.111	
		Two	GRE	28.618	0.815	
		Two	S8	30.192	1.110	
		Four	DEM 38	32.111	1.366	
		Four	MCK	34.326	1.126	
		DAY	C	One	45	30.880
One	SF			40.050	4.281	
Two	30			30.167	3.432	
Two	34			22.241	2.195	
Four	DEM 38			23.300	2.168	
Four	MCK			30.880	3.583	
S	One		41	53.636	3.010	
	One		60	50.591	3.431	
	Two		GRE	56.667	3.889	
	Two		S8	43.235	2.960	
	Four		DEM 38	45.333	2.331	
	Four		MCK	45.913	3.896	
	U		One	31.5	16.750	2.055
			One	33	20.100	1.755
Two			30	22.690	1.705	
Two			34	22.724	1.547	
Four			DEM 38	21.536	2.344	
Four			MCK	14.833	1.478	
X			One	BR	44.000	2.218
			One	LCWW	25.286	2.671
			Two	GRE	45.238	3.267
			Two	S8	42.263	2.669
	Four		DEM 38	37.600	3.652	
	Four	MCK	23.565	2.435		
HGT	C	One	45	50.768	3.064	
		One	SF	62.420	3.760	

(Table A.9 continues)

Table A.9 (continued)

<b>Trait</b>	<b>Species</b>	<b>Community Type</b>	<b>Community</b>	<b>Mean</b>	<b>SE</b>	
HGT	C	Two	30	53.367	4.463	
		Two	34	47.610	2.705	
		Four	DEM 38	50.030	3.446	
		Four	MCK	48.916	2.626	
	S	One	41	63.750	3.582	
		One	60	63.045	2.201	
		Two	GRE	65.558	3.907	
		Two	S8	61.482	4.028	
		Four	DEM 38	59.314	3.361	
		Four	MCK	63.275	3.232	
		U	One	31.5	66.754	3.863
			One	33	62.327	3.609
	Two		30	68.710	2.737	
	Two		34	72.976	3.389	
	Four		DEM 38	61.668	3.469	
	Four		MCK	59.279	3.182	
	X	One	BR	82.211	4.986	
		One	LCWW	63.636	4.160	
		Two	GRE	83.162	4.857	
		Two	S8	87.474	3.894	
Four		DEM 38	92.580	3.090		
Four		MCK	63.591	4.189		
PA		C	One	45	1.377	0.100
			One	SF	1.083	0.077
	Two		30	1.750	0.128	
	Two		34	2.312	0.146	
	Four		DEM 38	1.702	0.086	
	Four		MCK	1.393	0.094	
	S	One	41	2.844	0.190	
		One	60	2.807	0.172	
		Two	GRE	2.655	0.250	
		Two	S8	3.308	0.212	
		Four	DEM 38	3.441	0.153	
		Four	MCK	3.704	0.165	
		U	One	31.5	0.943	0.041
			One	33	1.190	0.053
	Two		30	0.976	0.037	
	Two		34	1.082	0.037	

(Table A.9 continues)

Table A.9 (continued)

<b>Trait</b>	<b>Species</b>	<b>Community Type</b>	<b>Community</b>	<b>Mean</b>	<b>SE</b>
PA	U	Four	DEM 38	0.939	0.040
		Four	MCK	0.870	0.036
	X	One	BR	1.812	0.137
		One	LCWW	1.729	0.089
		Two	GRE	1.468	0.067
		Two	S8	1.558	0.063
		Four	DEM 38	1.768	0.147
		Four	MCK	1.951	0.080
PC1	C	One	45	-0.131	0.008
		One	SF	-0.123	0.014
		Two	30	-0.120	0.023
		Two	34	-0.118	0.011
		Four	DEM 38	-0.116	0.010
		Four	MCK	-0.119	0.011
	S	One	41	-0.195	0.015
		One	60	-0.189	0.011
		Two	GRE	-0.179	0.018
		Two	S8	-0.204	0.011
		Four	DEM 38	-0.204	0.009
		Four	MCK	-0.204	0.012
	U	One	31.5	0.200	0.006
		One	33	0.192	0.006
		Two	30	0.210	0.006
		Two	34	0.202	0.007
		Four	DEM 38	0.201	0.006
		Four	MCK	0.222	0.006
	X	One	BR	0.003	0.015
		One	LCWW	0.042	0.013
		Two	GRE	0.026	0.010
		Two	S8	0.028	0.011
		Four	DEM 38	0.046	0.013
		Four	MCK	0.052	0.015
PC2	C	One	45	-0.003	0.009
		One	SF	0.006	0.015
		Two	30	0.004	0.025
		Two	34	0.005	0.009
		Four	DEM 38	0.001	0.012
		Four	MCK	0.008	0.009

(Table A.9 continues)

Table A.9 (continued)

<b>Trait</b>	<b>Species</b>	<b>Community Type</b>	<b>Community</b>	<b>Mean</b>	<b>SE</b>	
PC2	S	One	41	0.051	0.016	
		One	60	0.039	0.013	
		Two	GRE	0.040	0.018	
		Two	S8	0.060	0.017	
		Four	DEM 38	0.069	0.013	
		Four	MCK	0.057	0.014	
	U	One	31.5	0.068	0.011	
		One	33	0.137	0.010	
		Two	30	0.058	0.012	
		Two	34	0.078	0.014	
		Four	DEM 38	0.083	0.010	
		Four	MCK	0.049	0.011	
	X	One	BR	-0.201	0.017	
		One	LCWW	-0.231	0.011	
		Two	GRE	-0.225	0.010	
		Two	S8	-0.224	0.014	
		Four	DEM 38	-0.222	0.007	
		Four	MCK	-0.243	0.010	
	STY	C	One	45	11.625	0.461
			One	SF	10.115	0.380
Two			30	12.647	0.363	
Two			34	14.447	0.427	
Four			DEM 38	12.204	0.590	
Four			MCK	11.459	0.321	
S		One	41	11.977	0.536	
		One	60	11.290	0.404	
		Two	GRE	12.066	0.766	
		Two	S8	12.293	0.473	
		Four	DEM 38	11.314	0.680	
		Four	MCK	12.036	0.612	
U		One	31.5	14.801	0.501	
		One	33	18.783	0.462	
		Two	30	15.797	0.434	
		Two	34	18.599	0.518	
		Four	DEM 38	14.309	0.408	
		Four	MCK	13.787	0.511	
X		One	BR	13.695	0.863	
		One	LCWW	14.626	0.712	
	Two	GRE	13.345	0.400		
	Two	S8	12.802	0.427		
	Four	DEM 38	14.584	0.644		
	Four	MCK	13.449	0.507		

## APPENDIX B

### CHAPTER 2 APPENDIX

Table B.1. Names, species compositions, and locations of the 11 natural communities that served as seed sources for the experiment. For species present and species collected, C = *Clarkia cylindrica*, U = *Clarkia unguiculata*, S = *Clarkia speciosa*, and X = *Clarkia xantiana*.

<b>Community Name</b>	<b>Species present</b>	<b>Species collected</b>	<b>Latitude</b>	<b>Longitude</b>
Black Gulch	S, X	S, X	35.59117	-118.52815
Black Gulch Entrance	S	S	35.59493	-118.52420
Bodfish Lookout	S	S	35.58930	-118.50453
Borel	X	X	35.59102	-118.51789
China Gardens	X	X	35.53789	-118.64891
Corral 1518	S	S	35.84799	-118.45060
Democrat	C, U, S, X	S, X	35.52891	-118.62655
Erskine Creek	X	X	35.58986	-118.44528
GRC	S, X	S, X	35.59700	-118.50735
GRE	S, X	S, X	35.59714	-118.50563
Mill Creek	C, U, S, X	S, X	35.53633	-118.61418

Table B.2. Model selection results for models of the proportion of seeds set in *C. speciosa* and *C. xantiana* focal plants. The measure of reproductive success was the percentage of ovules that set seeds per flower per plant. Community type refers to the species composition of the site where the focal plant was placed during the experiment—blank, early species only, or mixed species composition. Period refers to the flowering period—early or late. The two random effects that were included in all models were the treatment site (the site where the experiment took place) and the source site (the seed source of the plant). Pseudo  $R^2$  values are reported for the best model (when a model was an improvement over the random effects only; pseudo  $R^2$  values are marginal  $R^2_{GLMM}$  values that represent the amount of variance explained by the fixed effects in the model. Log-likelihood ratio tests were used to assess the significance of terms in the models by comparing a given model to a model with one fewer fixed effect.

Species	Model #	Model specifications	AIC	Pseudo $R^2$	vs. Model #	$\chi^2$	df	<i>P</i>
<i>C. speciosa</i>	1	~Community Type*Period +(1 Treatment Site) +(1 Source Site)	-105.19		2	1.0504	2	0.5914
	2	~ Community Type+Period +(1 Treatment Site) +(1 Source Site)	-108.14		3	2.5444	1	0.1107
					4	1.3987	2	0.4969
	3	~ Community Type +(1 Treatment Site) +(1 Source Site)	-107.60		5	1.7515	2	0.4165
	4	~Period +(1 Treatment Site) +(1 Source Site)	-110.74		5	2.8973	1	0.08873
	5	~(1 Treatment Site) +(1 Source Site)	-109.85					
<i>C. xantiana</i>	1	~Community Type*Period +(1 Treatment Site) +(1 Source Site)	-223.84		2	0.3173	2	0.8533

(Table B.2 continues)

Table B.2 (continued)

Species	Model #	Model specifications	AIC	Pseudo R <sup>2</sup>	vs. Model #	$\chi^2$	df	P
<i>C. xantiana</i>	2	~Community Type+Period			3	32.98	1	9.311e-09
		+(1 Treatment Site) +(1 Source Site)	-227.53		4	1.7306	2	0.4209
	3	~Community Type			5	1.8715	2	0.3923
		+(1 Treatment Site) +(1 Source Site)	-196.55					
	4	~Period			5	33.121	1	8.66e-09
	+(1 Treatment Site) +(1 Source Site)	<b>-229.80</b>	<b>0.0897</b>					
	5	~(1 Treatment Site) +(1 Source Site)	-198.68					

Table B.3. Estimated marginal means for the average proportion of seeds per plant for *C. xantiana* plants at each community type and during both of the experimental flowering periods. Lower CI and Upper CI indicate the bounds of 95% confidence intervals. Contrasts indicate whether seed set differed between the late and early period at a given community type. A negative contrast indicates that plants set more seeds per flower during the early flowering period.

Species	Community Type	Period	Estimated marginal means				Contrasts		
			Estimate	SE	Lower CI	Upper CI	Estimate $\pm$ SE	t ratio	P value
<i>C. xantiana</i>	Blank	Early	0.443	0.076	0.259	0.627	-0.111 $\pm$	-3.245	0.0013
		Late	0.332	0.076	0.148	0.516	0.0341		
	Early sp. only	Early	0.563	0.063	0.420	0.705	-0.145 $\pm$	-3.068	0.0024
		Late	0.418	0.065	0.272	0.564	0.0471		
	Mixed	Early	0.524	0.062	0.373	0.674	-0.126 $\pm$	-4.475	< 0.0001
		Late	0.398	0.062	0.247	0.548	0.0281		

Table B.4. Linear mixed model selection results for models of the pollinator visitation rates to *C. speciosa* and *C. xantiana* focal plants. Community type refers to the species composition of the site where the focal plant was placed during the experiment—blank, early species only, or mixed species composition. Period refers to the flowering period—early or late. The random effect that was included in all models was the treatment site (the site where the experiment took place). Log-likelihood ratio tests were used to assess the significance of terms in the models by comparing a given model to a model with one fewer fixed effect.

Species	Model #	Model specifications	AIC	Log-likelihood ratio tests			
				vs. Model #	$\chi^2$	df	<i>P</i>
<i>C. speciosa</i>	1	~Community Type*Period + (1 Treatment Site)	48.612	2	1.0316	2	0.597
	2	~Community Type+Period + (1 Treatment Site)	45.644	3	0.6776	1	0.410
				4	7.4004	2	0.025
	3	~Community Type + (1 Treatment Site)	44.321	5	7.2962	2	0.026
	4	~Period + (1 Treatment Site)	49.044	5	0.5735	1	0.449
5	~(1 Treatment Site)	47.618					
<i>C. xantiana</i>	1	~Community Type*Period + (1 Treatment Site)	81.877	2	0.5064	2	0.7763
	2	~Community Type + Period + (1 Treatment Site)	78.383	3	0.0305	1	0.8613
				4	1.126	2	0.5695
	3	~Community Type + (1 Treatment Site)	76.414	5	1.1253	2	0.5697
	4	~Period +(1 Treatment Site)	75.509	5	0.0299	1	0.8627
5	~(1 Treatment Site)	73.539					

Table B.5. Linear mixed model selection results for models of the site floral densities at sites that contained *C. speciosa* and *C. xantiana* focal plants. Community type refers to the species composition of the site where the focal plant was placed during the experiment—early species only, or mixed species composition. Period refers to the flowering period—early or late. The random effect that was included in all models was the treatment site (the site where the experiment took place). Log-likelihood ratio tests were used to assess the significance of terms in the models by comparing a given model to a model with one fewer fixed effect.

Species	Model #	Model specifications	AIC	Log-likelihood ratio tests			
				vs. Model #	$\chi^2$	df	P
<i>C. speciosa</i>	1	~Community Type*Period + (1 Treatment Site)	82.647	2	1.4472	2	0.229
	2	~Community Type + Period + (1 Treatment Site)	82.094	3	3.5218	1	0.061
				4	2.1442	1	0.143
	3	~Community Type + (1 Treatment Site)	83.616	5	1.6341	2	0.201
	4	~Period +(1 Treatment Site)	82.239	5	3.0117	1	0.083
5	~(1 Treatment Site)	83.250					
<i>C. xantiana</i>	1	~Community Type*Period + (1 Treatment Site)	51.237	2	0.0014	1	0.9701
	2	~Community Type + Period + (1 Treatment Site)	49.238	3	2.6824	1	0.1015
				4	0.0718	1	0.7888
	3	~Community Type + (1 Treatment Site)	49.921	5	0.1022	1	0.7492
	4	~Period +(1 Treatment Site)	47.310	5	2.7128	1	0.0996
5	~(1 Treatment Site)	48.023					

Table B.6. Model selection results for models of the proportion of seeds set in *C. speciosa* and *C. xantiana* focal plants as a function of pollinator visitation rate, site floral density, and their interaction. The measure of reproductive success was the percentage of ovules that set seeds per flower per plant. The two random effects that were included in all models were the treatment site (the site where the experiment took place) and the source site (the seed source of the plant). Pseudo R<sup>2</sup> values are reported for the best model (when a model was an improvement over the random effects only; pseudo R<sup>2</sup> values are marginal R<sup>2</sup>GLMM values that represent the amount of variance explained by the fixed effects in the model. Log-likelihood ratio tests were used to assess the significance of terms in the models by comparing a given model to a model with one fewer fixed effect.

Species	Model #	Model specifications	AIC	Pseudo R <sup>2</sup>	vs. Model #	$\chi^2$	df	P
<i>C. speciosa</i>	1	~Pollinator Visitation Rate *Density +(1 Treatment Site) +(1 Source Site)	-110.37		2	6.2433	1	0.01247
	2	~ Pollinator Visitation Rate+Density +(1 Treatment Site) +(1 Source Site)	-106.13		3	0.065	1	0.7987
					4	0.2827	1	0.5949
	3	~ Pollinator Visitation Rate +(1 Treatment Site) +(1 Source Site)	-108.06		5	0.2178	1	0.6407
	4	~Density +(1 Treatment Site) +(1 Source Site)	-107.85		5	0.0001	1	0.9923
	5	~(1 Treatment Site) +(1 Source Site)	-109.85					

(Table B.6 continues)

Table B.6 (continued)

Species	Model #	Model specifications	AIC	Pseudo R <sup>2</sup>	vs. Model #	$\chi^2$	df	P
<i>C. xantiana</i>	1	~Pollinator Visitation Rate						
		*Density						
		+(1 Treatment Site) +(1 Source Site)	-203.81		2	1.8416	1	0.1748
	2	~ Pollinator Visitation Rate+Density						
		+(1 Treatment Site) +(1 Source Site)	-203.97		3	9.2922	1	0.002301
	3	~ Pollinator Visitation Rate						
		+(1 Treatment Site) +(1 Source Site)	-196.68		5	0.0001	1	0.9855
	4	~Density						
		+(1 Treatment Site) +(1 Source Site)	<b>-205.95</b>	<b>0.0695</b>	<b>5</b>	<b>9.2792</b>	<b>1</b>	<b>0.002318</b>
	5	~(1 Treatment Site) +(1 Source Site)	-198.68					

APPENDIX C

CHAPTER 3 APPENDIX

Table C.1. Source communities utilized in the common garden. For species composition, *C* = *C. cylindrica*, *U* = *C. unguiculata*, *S* = *C. speciosa*, and *X* = *C. xantiana*. Floral mass data are the average  $\pm$  1 SE of 20 flowers (10 male and 10 female) from that community.

Community Name	Latitude	Longitude	Species composition	Floral wet mass (g)	Floral dry mass (g)
Site 33	35.4657	-118.7538	<i>U</i>	0.127595 $\pm$ 0.006931	0.032200 $\pm$ 0.001976
Green Corner	35.4615	-118.7627	<i>U</i>	0.122480 $\pm$ 0.007222	0.026620 $\pm$ 0.001713
Callbox 323	35.5534	-118.6158	<i>U</i>	0.100425 $\pm$ 0.008205	0.021675 $\pm$ 0.002006
Main Highway Gulley	35.5834	-118.5302	<i>C</i>	0.092420 $\pm$ 0.006989	0.019645 $\pm$ 0.001506
Upper Coyote Gulch	35.5816	-118.5216	<i>C</i>	0.095650 $\pm$ 0.005825	0.020295 $\pm$ 0.001141
Sandy Flats	35.5809	-118.5258	<i>C</i>	0.096650 $\pm$ 0.006690	0.020040 $\pm$ 0.001265
Summer Camp	35.5294	-118.6460	<i>U</i>	0.085860 $\pm$ 0.004403	0.016955 $\pm$ 0.001074
			<i>C</i>	0.132430 $\pm$ 0.008060	0.027980 $\pm$ 0.001578
North Pole	35.5323	-118.6472	<i>U</i>	0.092390 $\pm$ 0.004091	0.019445 $\pm$ 0.000811
			<i>C</i>	0.087565 $\pm$ 0.005921	0.019060 $\pm$ 0.001180
Divot	35.4742	-118.7286	<i>U</i>	0.089840 $\pm$ 0.005520	0.020970 $\pm$ 0.001344
			<i>C</i>	0.148845 $\pm$ 0.010543	0.029585 $\pm$ 0.002052
Democrat	35.5289	-118.6266	<i>U</i>	0.113970 $\pm$ 0.004510	0.025620 $\pm$ 0.001087
			<i>C</i>	0.101840 $\pm$ 0.005847	0.020300 $\pm$ 0.001383
Delonegha East	35.5464	-118.6170	<i>S</i>		
			<i>X</i>		
Delonegha East	35.5464	-118.6170	<i>U</i>	0.091820 $\pm$ 0.006289	0.020935 $\pm$ 0.001475
			<i>C</i>	0.075940 $\pm$ 0.005110	0.015205 $\pm$ 0.000982
Mill Creek	35.5363	-118.6142	<i>S</i>		
			<i>X</i>		
Mill Creek	35.5363	-118.6142	<i>U</i>	0.085310 $\pm$ 0.004255	0.018130 $\pm$ 0.001303
			<i>C</i>	0.101405 $\pm$ 0.006916	0.020550 $\pm$ 0.001565

## Appendix C-1: Supplemental methods and results from qualitative SPME sampling

*Rationale.* We conducted two types of additional analyses using Solid Phase Micro Extraction (SPME) fibers (Supelco, Inc., (Sigma-Aldrich), Bellefonte, PA). To determine if the presence of additional flowers changed the composition of the volatile profile (i.e. due to threshold effects), we compared the profiles of samples with three versus six cut flowers from the same plant. To determine if volatiles are produced in the petals and/or reproductive parts of these flowers, we compared the volatile profiles of dissected petals from six flowers versus those of the remaining tissues of the same six flowers.

*Methods.* We used the 65  $\mu$  “Stabilflex” field-assembly fibers (with both divinylbenzene and polydimethylsiloxane in the adsorbent matrix) because of their proven versatility in trapping different biosynthetic classes of volatile compounds (Goodrich & Raguso, 2009). Both types of collections were performed on plants of both species from one one-species community (*C. cylindrica*: MHG; *C. unguiculata*: CB323) and from one two-species community (SC for both species). Three replicates of each comparison were sampled for each analysis (e.g. 3 samples with 3 flowers and 3 samples with 6 flowers from each community for each species).

Samples were collected in 20 mL glass scintillation vials sealed with Nalophan (PTE) film (Toppits®, Cofresco Frischhalteprodukte, Minden, Germany). One ambient control vial was sampled during each sampling period. All samples were equilibrated for 60 minutes, and exposed to a SPME fiber for 30 minutes. After the exposure period, fibers were inserted into the GC injection port for thermal desorption. Analysis via GC-MS followed the same method used for the solvent-eluted samples.

Peak areas were integrated manually using Shimadzu GCMSolutions software. We

observed 45 compounds across all samples (Table C.2), and compounds were identified using the same protocols as the solvent-eluted samples (see Methods). To exclude experimental artifacts, each sample was compared to the concurrently collected ambient control. No ambient control samples contained the compounds detected in the floral samples.

*Statistical analysis.* To determine if the presence of additional flowers changed the composition of the volatile profile (i.e. due to threshold effects), we compared the number of compounds observed in the three flower samples versus the six flower samples. Specifically, we calculated the total number of compounds in each sample, and the number of monoterpenoids, sesquiterpenoids, aromatics, and “green leafy volatiles” (see Table C.2). To test for differences in these count data, we ran paired Wilcoxon signed rank tests for each compound class.

To determine if volatiles are produced in the petals and/or reproductive parts of these flowers, we compared the presences and absences of all compounds across petal and non-petal samples. We performed a Permutational Multivariate Analysis of Variance (PERMANOVA) using a jaccard distance matrix on the jaccard dissimilarity values between samples using the *adonis* function from the *vegan* package in R (Oksanen et al., 2019). We visualized the differences between the petal and non-petal samples using the multivariate dispersion, which shows the average distance to the group centroid.

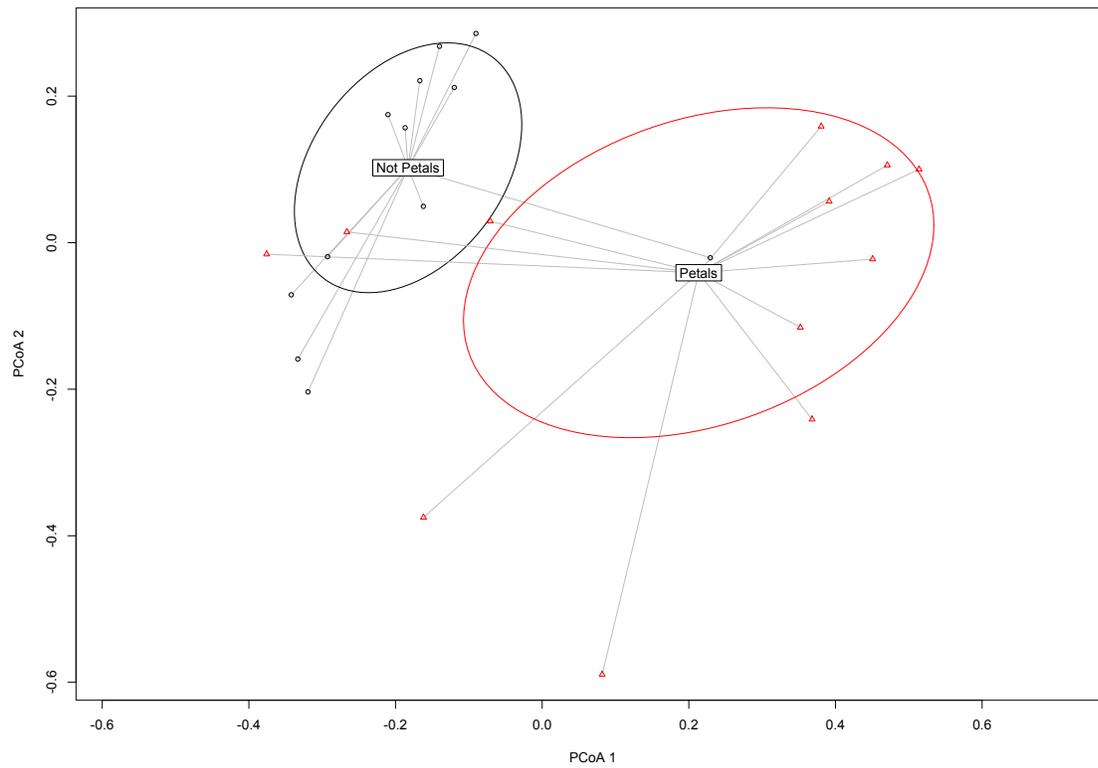
*Results.* Samples with six flowers contained significantly more compounds than samples with three flowers ( $Z = -2.4382$ ,  $P = 0.015$ ). Specifically, the samples with six flowers contained more monoterpenoids ( $Z = -2.4945$ ,  $P = 0.013$ ), and sesquiterpenoids ( $Z = -2.1264$ ,  $P = 0.033$ ). In the PERMANOVA analysis of samples from petals and non-petals, there was a significant effect of sample type ( $R^2 = 0.1555$ ,  $P = 0.002$ ). Petal and non-petal samples formed two distinct clusters based on their multivariate dispersion (Figure C.1). Non-petal samples contained significantly

more compounds than petal samples ( $Z = 3.0618$ ,  $P = 0.0022$ ).

*Conclusions.* The significant increase in monoterpenoids and sesquiterpenoids in the samples with six flowers suggests that increasing the floral tissue in a sample can increase the probability of detecting a fuller complement of compounds, more representative of a blooming inflorescence. As such, we collected quantitative samples from plants with six or more open flowers in our study. The dissected flower tissues were separated in multivariate space, which suggests that scent is differentially produced across types of floral tissue in both species. In particular, the non-petal samples contained more compounds than the petal samples, which suggests that increases in volatile production may not be strongly correlated with increases in petal size in these species.

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**Figure C.1. Multivariate dispersion of the petal and non-petal samples, performed using jaccard distances. The ellipses show the group standard deviations, and lines represent the average distance to the group centroid.**



Table C.2 (continued)

Class	Class notes	Present in dynamic headspace	cylindrica headspace samples	unguiculata headspace samples	Present in SPME	Compound name	IUPAC	RT, min.	KI	Published KI	Standard run	Percent match with library	Mass spectra for tentatively identified compounds and unknowns:
Monoterpenoids	unsaturated monoterpene	x	16	49 x	x	2,6-dimethyl-1,3,5,7-octatetraene (trans)	(3E,5E)-2,6-dimethylocta-1,3,5,7-tetraene	8.457	1466		N	<90% (low 80s)	41 (51.2), 55 (28.2), 57 (100), 65 (20.7), 77 (38.6), 79 (31.4), 91 (96.8), 105 (19.5), 119 (63.4), 134 (35.0)
	irregular terpeneoid	x	41	36 x	x	6-methyl-5-hepten-2-one	6-methylhept-5-en-2-one	7.408	1356	1319	N	96%	41 (51.2), 43 (100), 53 (7.6), 55 (32.2), 58 (14.7), 69 (21.9), 93 (7.6), 108 (18.9), 111 (7.4), 126 (2.0)
		x	8	68 x	x	alpha pinene	4,6,6-trimethylbicyclo[3.1.1]hept-3-ene	4.009	1013	1092; 1034	Y		
		x	20	16 x	x	alpha terpinene	1-methyl-4-propan-2-ylcyclohexa-1,3-diene	5.296	1155	1178	N	93%	41 (43.2), 43 (19.4), 53 (19.9), 77 (51.6), 79 (50.2), 91 (56.6), 93 (100), 105 (23.0), 121 (70.6), 136 (33.6)
	Terpene alcohol	x	27	30 x	x	alpha terpineol	2-(4-methylcyclohex-3-en-1-yl)propan-2-ol	10.648	1722	1661; 1669	Y		
		x	3	10 x	x	alpha terpinolene	1-methyl-4-propan-2-ylidenecyclohexene	6.865	1302	1275	N	<90%	45 (56.3), 51 (8.8), 58 (75.8), 77 (43.5), 79 (45.3), 91 (53.0), 93 (100), 105 (19.1), 121 (60.7), 136 (49.1)
		x	43	86 x	x	beta myrcene	7-methyl-3-methylideneocta-1,6-diene	5.581	1169	1166; 1161	Y		
		x	47	44 x	x	beta phellandrene	3-methylidene-6-propan-2-ylcyclohexene	6.065	1227	1209	N	95%	41 (20.7), 65 (11.4), 77 (43.2), 79 (24.9), 80 (11.5), 91 (47.2), 92 (11.6), 93 (100), 94 (14.0), 136 (15.6)
		x	24	68 x	x	beta pinene	6,6-dimethyl-4-methylidenebicyclo[3.1.1]heptane	4.942	1111	1136; 1114	Y		
	Terpene alcohol	x	9	66	x	borneol	4,7,7-trimethylbicyclo[2.2.1]heptan-3-ol	9.468	1579	1677	N	<90%	45 (30.8), 53 (28.6), 67 (31.8), 77 (53.2), 79 (52.7), 81 (31.5), 91 (68.1), 93 (70.0), 95 (100), 150 (38.6)
		x	64	96 x	x	cis beta ocimene	(3Z)-3,7-dimethylocta-1,3,6-triene	6.346	1253	1225	Y		
		x	19	39 x	x	gamma terpinene	1-methyl-4-propan-2-ylcyclohexa-1,4-diene	6.474	1261	1238	Y		
	Terpene alcohol	x	43	14	x	geraniol	(2E)-3,7-dimethylocta-2,6-dien-1-ol	10.492	1702	1814	N	90%	41 (100), 53 (10.1), 59 (8.6), 67 (14.9), 68 (18.6), 69 (76.9), 81 (7.2), 93 (5.9), 111 (17.8), 123 (6.8)
		x	35	45 x	x	limonene	1-Methyl-4-(prop-1-en-2-yl)cyclohex-1-ene	5.951	1300	1217	Y		
	Terpene alcohol	x	14	21 x	x	linalool	3,7-Dimethylocta-1,6-dien-3-ol	9.338	1563	1517; 1522	Y		
	Oxygenated	x	26	33 x	x	myroxide	2,2-dimethyl-3-[(2E)-3-methylpenta-2,4-dienyl]oxirane	8.831	1506		N	<90% (low 80s)	41 (22.4), 43 (53.3), 53 (13.4), 56 (40.0), 73 (18.2), 77 (27.7), 79 (100), 81 (38.3), 84 (12.9), 93 (11.1)
	monoterpene aromatic	x	0	23 x	x	para cymene	1-methyl-4-propan-2-ylbenzene	6.725	1282	1261	Y		
		x	26	52 x	x	sabinene	4-methylidene-1-propan-2-ylbicyclo[3.1.0]hexane	5.103	1116	1123	Y		
	Terpene alcohol	x	1	22 x	x	sabinene hydrate	4-methyl-1-propan-2-ylbicyclo[3.1.0]hexan-4-ol	9.400	1571		N	90%	43 (100), 45 (14.7), 53 (11.8), 69 (33.6), 71 (41.7), 79 (22.8), 81 (31.1), 93 (28.0), 111 (19.6), 121 (13.2)
	Terpene alcohol	x	0	10 x	x	terpinen-4-ol	4-methyl-1-propan-2-ylcyclohex-3-en-1-ol	9.883	1628	1579	Y		
		x	67	103 x	x	trans beta ocimene	(3E)-3,7-dimethylocta-1,3,6-triene	6.529	1274	1250; 1242	Y		
	Oxygenated	x	0	4 x	x	verbenone	2,6,6-trimethylbicyclo[3.1.1]hept-2-en-4-one	10.804	1741		N	90%	41 (67.1), 53 (39.0), 55 (36.8), 67 (42.9), 77 (38.0), 79 (53.0), 80 (61.6), 91 (77.1), 107 (100), 135 (51.6)

(Table C.2 continues)

Table C.2 (continued)

Class	Class notes	Present in dynamic headspace	cylindrica headspace samples	unguiculata headspace samples	Present in SPME	Compound name	IUPAC	RT, min.	KI	Published KI	Standard run	Percent match with library	Mass spectra for tentatively identified compounds and unknowns:
Sesquiterpenes and C15 derivatives		x	47	22	x	alloaromadendrene	(4aS,7R,7aR)-1,1,7-trimethyl-4-methylidene-2,3,4a,5,6,7,7a,7b-octahydro-1aH-cyclopropa[e]azulene	10.365	1669	1639	Y		
		x	46	15	x	alpha bergamotene	4,6-dimethyl-6-(4-methylpent-3-enyl)bicyclo[3.1.1]hept-3-ene	9.783	1616		N	93%	41 (72.4), 55 (43.2), 69 (36.8), 77 (35.9), 79 (35.2), 91 (44.5), 93 (100), 105 (25.5), 107 (30.4), 119 (83.8)
		x	65	42	x	alpha humulene	(1E,4E,8E)-2,6,6,9-tetramethylcycloundeca-1,4,8-triene	10.528	1699	1641; 1663	Y		
		x	132	14	x	beta cadinene	(1S,4aR,8aS)-4,7-dimethyl-1-propan-2-yl-1,2,4a,5,8,8a-hexahydronaphthalene	10.693	1713		Y		
		x	9	6		beta farnesene	(6E)-7,11-dimethyl-3-methylidenedodeca-1,6,10-triene	10.352	1678	1658	Y		
		x	85	34	x	beta longipinene	(1S,2R,7S,8S)-2,6,6-Trimethyl-9-methylenetricyclo[5.4.0.0.2,8]undecane	10.925	1757		N	<90%	41 (100), 67 (36.1), 79 (69.2), 91 (65.9), 93 (59.4), 105 (54.12), 107 (55.4), 119 (36.8), 133 (37.2), 161 (64.8)
	Oxygenated	x	38	7		caryophyllene oxide	(1R,4R,6R,10S)-9-Methylene-4,12,12-trimethyl-5-oxatricyclo[8.2.0.0 <sup>4,6</sup> ]dodecane	12.928	2029	1962	N	<90% (low 80s)	41 (100), 67 (37.2), 77 (45.6), 79 (77.7), 91 (53.0), 93 (66.5), 95 (43.6), 96 (36.2), 105 (40.0), 107 (33.4)
		x	16	9	x	cis DMNT	(3Z)-4,8-dimethylnona-1,3,7-triene	6.777	1293		N	93%	41 (100), 53 (14.9), 67 (8.9), 69 (96.7), 79 (12.5), 81 (9.7), 82 (6.5), 94 (4.3), 107 (7.7), 135 (2.6)
		x	4	39	x	gamma cadinene	(1S,4aR,8aR)-7-methyl-4-methylidene-1-propan-2-yl-2,3,4a,5,6,8a-hexahydro-1H-naphthalene	11.199	1791	1759; 1752	N	<90%	41 (81.5), 55 (36.28), 81 (48.7), 91 (63.6), 105 (83.5), 119 (90.9), 134 (90.4), 161 (100), 189 (26.1), 204 (33.0)
		x	59	67	x	germacrene D	(1E,6E)-1-methyl-5-methylidene-8-propan-2-ylcyclodeca-1,6-diene	10.885	1737	1687; 1705	Y		
	Oxygenated	x	126	10	x	intermedeol	(1S,4aS,7R,8aS)-1,4a-dimethyl-7-prop-1-en-2-yl-2,3,4,5,6,7,8,8a-octahydronaphthalen-1-ol	14.560	2278		N	95%	41 (52.8), 43 (100), 55 (29.8), 67 (32.7), 71 (29.0), 81 (37.9), 93 (19.8), 161 (19.9), 189 (19.5), 204 (19.1)
		x	6	1		patchoulane	1H-3a,7-Methanoazulene, octahydro-1,4,9,9-tetramethyl-	13.994	2189		N	<90% (low 80s)	41 (100), 53 (42.8), 55 (54.8), 67 (45.8), 79 (44.4), 91 (50.5), 93 (38.1), 95 (38.2), 105 (39.1), 119 (32.7)
		x	63	73	x	trans beta caryophyllene	(1R,4E,9S)-4,11,11-trimethyl-8-methylidenebicyclo[7.2.0]undec-4-ene	9.920	1626	1575; 1594	Y		
		x	1	20		trans DMNT	(3E)-4,8-dimethylnona-1,3,7-triene	7.106	1319		Y		
	x	26	53	x	trans trans alpha farnesene	(3E,6E)-3,7,11-trimethyldodeca-1,3,6,10-tetraene	11.068	1762	1725	Y			
	x	1	12	x	unknown C15H24 (1)		10.671	1724		N	NA	43 (86.0), 57 (100), 71 (70.6), 77 (13.7), 79 (12.9), 85 (27.4), 93 (27.8), 99 (12.0), 105 (19.6), 161 (26.2)	
	x	66	4	x	unknown C15H24 (2)		10.927	1757		N	NA	41 (100), 77 (43.8), 79 (61.1), 91 (66.2), 93 (57.3), 105 (57.9), 107 (47.2), 119 (40.2), 133 (31.3), 161 (57.5)	
	x	113	15	x	unknown C15H24 (3)		11.283	1799		N		41 (81.2), 55 (44.0), 79 (38.2), 81 (37.0), 91 (48.1), 93 (30.6), 105 (38.6), 107 (75.8), 122 (88.0), 161 (100)	

## Appendix C-2: Supplemental methods and results from tests for wounding artifacts

*Rationale.* We observed differences across species and community types in compounds that are generally considered “green leafy volatiles” associated with plant wounding (Visser and Ave 1978; Scala et al. 2013). To determine if these patterns were the product of an experimental artifact, we conducted a second greenhouse common garden experiment to compare the volatiles emitted by wounded and non-wounded plants.

*Plant source community selection.* We grew individuals of both species from seeds from one single-species community per species (*C. cylindrica*: MHG; *C. unguiculata*: GRCO), one two-species community per species (SC for both species), and one four-species community per species (MCK for both species). These communities were specifically selected to represent a range in the emission rates of the “green leafy volatiles” observed in the 2018 common garden.

*Plant germination and growth.* Seeds were stratified and transplanted in two batches in January and February of 2019. All procedures were identical to those used in the 2018 common garden.

*Quantitative scent analysis.* Floral volatile samples were collected using the dynamic headspace adsorption technique between March 21, 2019 and April 6, 2019. All collection protocols were identical to those used in the 2018 common garden. To assess the potential for elevated emission of “green leafy volatiles” due to wounding, each plant was sampled twice. On the first sampling day for a given plant, we sampled the floral bouquet using the same methods as in 2018, to produce a control sample. On the second sampling day for a given plant, we sampled the floral bouquet and inflicted wounds to the plant immediately prior to the collection window. Using scissors, we snipped a piece of each leaf included in the collection chamber for *C. cylindrica*, and we snipped every other leaf included in the collection chamber for *C. unguiculata* because

*C. unguiculata* plants have more, larger leaves. This resulted in a mean  $\pm$  1 SE of  $0.065 \pm 0.005$  g of fresh leaf mass removed from *C. cylindrica* and  $0.049 \pm 0.004$  g of fresh leaf mass removed from *C. unguiculata*. The number of flowers included in each sample was recorded. We collected both non-wounded and wounded samples from 10 plants per species per community ( $N_{\text{total}} = 120$  samples).

*Scent analysis via GC-FID.* Samples were analyzed using a Shimadzu GC-FID (GC 2014) with an AOC-20i auto injector. One  $\mu\text{L}$  aliquots of the solvent eluted samples were injected (splitless mode) at 240C onto a polar GC column (EC Wax, 30m long, 0.25 mm internal diameter, 0.25 $\mu$  film thickness; BGB Analytik). The GC oven program (a 2-minute hold at 40C, followed by a 14.67C increase per minute to the maximum temperature of 260C, with a 2-minute hold at the maximum temperature) was optimized to minimize run length (for over 120 samples) while allowing for peak resolution to baseline. The “green leafy volatile” compounds of interest ((*Z*)-3-hexen-1-ol and (*Z*)-3-hexenyl acetate) were identified via direct comparison of retention time and mass spectra with those of authentic standards. Peak areas were automatically integrated by Shimadzu Postrun Analysis software.

*Extraction and processing of quantitative data.* Emission rates were calculated as in the 2018 common garden using response factors generated using external standard dose-response curves generated from log- and semi-log dilutions of the compounds of interest. Emission rates were related to floral masses using the floral mass data collected in 2018.

Emission rates from each sample were compared to the ambient control collected on that day, but none of the ambient control samples contained the compounds of interest.

*Statistical analysis.* The data were subset by species and we used paired t-tests (t.test function in R) to compare the wounded vs. non-wounded volatile emission profiles. We then examined the

2018 common garden data that had been cleaned to remove compounds with emission rates less than 5x the emission rates in the ambient control, but that had not been cleaned to remove compounds that occur in the vegetative controls. We analyzed this form of the 2018 data because we did not utilize vegetative controls in our 2019 common garden, such that the 2018 profiles prior to vegetative control screening are directly comparable to the 2019 profiles. We ran two sets of two sample t-tests for each species: 1) we compared the 2018 profiles to the 2019 non-wounded control samples, and 2) we compared the 2018 profiles to the 2019 wounded samples. Due to the variance structure of these data, we ran two sample t tests with unequal variance for (Z)-3-hexen-1-ol and with equal variances for (Z)-3-hexenyl acetate).

*Expected results.* If our experimental protocol induces a wounding response, we expect that the “green leafy volatile” emission rates will be equivalent across the 2019 wounded and 2019 non-wounded plants. If our experimental protocol does not induce a wounding response, we expect that the “green leafy volatile” emission rates will be higher in the 2019 wounded samples relative to the 2019 non-wounded samples. In addition, we expect that the 2018 emission rates will be roughly equivalent to the 2019 non-wounded emission rates, and lower than the 2019 wounded emission rates.

*Results and Discussion.* For both species and both compounds, emission rates were higher in the 2019 wounded samples relative to the 2019 non-wounded samples (Table C.3), which suggests that wounding elevates emission rates of these volatile compounds. For both species and compounds, emission rates did not differ between the 2019 control samples and the 2018 samples (see 2018 – 2019 C comparisons Table C.4), which suggests that our 2019 control samples are representative of the emission rates observed in 2018. For *C. unguiculata*, emissions rates of both compounds were lower in the 2018 samples relative to the 2019 wounded samples

(see 2018 – 2019 W comparisons Table C.4). Comparisons of emission rates for *C. cylindrica* yield similar patterns, although the comparison for (Z)-3-hexen-1-ol is non-significant but in the expected direction. Taken together, these results suggest that while wounding elevates the emission rates of these “green leafy volatiles,” the emission rates observed in 2018 cannot be attributed to wounding. Rather, these emission rates likely reflect constitutive emission of these compounds by flowering plants, which has been documented in other systems (e.g., Brodmann et al. 2008, 2012).

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Table C.3. Results of paired t-tests comparing the 2019 wounded and non-wounded plant samples. Ung refers to *C. unguiculata* and Cyl refers to *C. cylindrica*. CI.low and CI.high represent the lower and upper bounds respectively of a 95% confidence interval on the mean difference. Positive mean differences indicate higher emission rates in the wounded samples. Significant tests are bolded, and marginally significant tests are italicized.

Species	Compound	<i>t</i>	df	<i>P</i>	CI.low	CI.high	Mean difference
Ung	( <i>Z</i> )-3-hexen-1-ol	<i>1.7066</i>	29	<i>0.0986</i>	-0.0020	0.0226	0.0103
	( <i>Z</i> )-3-hexenyl acetate	<b>2.5461</b>	<b>29</b>	<b>0.0164</b>	0.0264	0.2421	0.1342
Cyl	( <i>Z</i> )-3-hexen-1-ol	<b>3.7503</b>	<b>29</b>	<b>0.0008</b>	0.0042	0.0145	0.0094
	( <i>Z</i> )-3-hexenyl acetate	<b>2.9732</b>	<b>29</b>	<b>0.0059</b>	0.0337	0.1825	0.1081

Table C.4. Results of two sample t-tests comparing the 2018 samples to the 2019 non-wounded controls (2019 C) and to the 2019 wounded samples (2019 W). Ung refers to *C. unguiculata* and Cyl refers to *C. cylindrica*. CI.low and CI.high represent the lower and upper bounds respectively of a 95% confidence interval on the mean difference. Mean 2019 refers to the mean of the 2019 group included in the specific analysis. Significant negative *t* values indicate that the 2019 group included in the test had a higher emission rate than the 2018 samples. Significant tests are bolded.

Species	Compound	Comparison	<i>t</i>	df	<i>P</i>	CI.low	CI.high	Mean 2018	Mean 2019
Ung	(Z)-3-hexen-1-ol	2018 – 2019 C	-1.4014	32	0.1707	-0.0209	0.0039	0.0102	0.0188
		2018 – 2019 W	<b>-5.0833</b>	<b>38</b>	<b>&lt; 0.0001</b>	-0.0263	-0.0113		0.0290
	(Z)-3-hexenyl acetate	2018 – 2019 C	-0.0258	73	0.9795	-0.1157	0.1127	0.2022	0.2036
		2018 – 2019 W	<b>-2.6321</b>	<b>73</b>	<b>0.0104</b>	-0.2385	-0.0321		0.3379
Cyl	(Z)-3-hexen-1-ol	2018 – 2019 C	0.6783	73	0.4997	-0.0058	0.0117	0.0156	0.0126
		2018 – 2019 W	-1.4618	73	0.1481	-0.0152	0.0023		0.0220
	(Z)-3-hexenyl acetate	2018 – 2019 C	-1.3750	73	0.1733	-0.1316	0.0241	0.1633	0.2170
		2018 – 2019 W	<b>-3.9411</b>	<b>73</b>	<b>0.0002</b>	-0.2437	-0.0800		0.3251

Table C.5. The 54 compounds in the dataset and their correlations with capscale axes 1 and 2. The *P* values (corrected for multiple tests, see *Materials and Methods*) are given for each correlation, as well as a column that lists the significance of each correlation at  $\alpha = 0.01$ . The Trans. column give the type of transformation that was applied for subsequent univariate analyses: log = log-transformation, sqrt = square root-transformation, none = no transformation.

Compound	Cor. with cap1	<i>P</i> cap1	Sig. cap1	Cor. with cap2	<i>P</i> cap2	Sig. cap2	Trans.
1 hexanol	0.1045	1.38E-01	No	-0.0613	4.86E-01	No	NA
2 dimethyl 1 3 5 7 octatetraene trans	0.3590	7.39E-09	Yes	0.3291	4.12E-07	Yes	log
2 amino phenyl ethanone	0.4807	4.60E-16	Yes	0.2602	1.01E-04	Yes	sqrt
2 phenyl ethanol	0.1233	7.99E-02	No	-0.0370	6.85E-01	No	NA
6 methyl 5 hepten 2 one	-0.1424	4.15E-02	No	-0.0624	4.86E-01	No	NA
alloaromadendrene	0.0035	9.55E-01	No	0.1265	1.20E-01	No	NA
$\alpha$ -bergamotene	-0.1897	4.71E-03	Yes	-0.0012	9.99E-01	No	log
$\alpha$ -humulene	-0.0561	4.40E-01	No	-0.0138	9.24E-01	No	NA
$\alpha$ -pinene	0.2016	2.75E-03	Yes	-0.2518	1.54E-04	Yes	log
$\alpha$ -terpinene	0.0284	6.93E-01	No	-0.1153	1.37E-01	No	NA
$\alpha$ -terpineol	0.1121	1.11E-01	No	-0.0618	4.86E-01	No	NA
$\alpha$ -terpinolene	0.1032	1.40E-01	No	-0.1162	1.37E-01	No	NA
benzyl acetate	0.0628	3.82E-01	No	0.0094	9.48E-01	No	NA
benzyl alcohol	-0.1679	1.46E-02	No	-0.0469	6.29E-01	No	NA
$\beta$ -cadinene	-0.6415	2.87E-31	Yes	0.0223	8.39E-01	No	none
$\beta$ -farnesene	0.0893	2.12E-01	No	0.0432	6.38E-01	No	NA
$\beta$ -longipinene	-0.3758	1.18E-09	Yes	0.0307	7.52E-01	No	sqrt
$\beta$ -myrcene	0.2861	6.79E-06	Yes	-0.0297	7.52E-01	No	sqrt
$\beta$ -phellandrene	0.0344	6.32E-01	No	-0.1224	1.20E-01	No	NA
$\beta$ -pinene	0.1980	3.22E-03	Yes	-0.2580	1.06E-04	Yes	log
borneol	0.3563	9.02E-09	Yes	0.3131	1.62E-06	Yes	sqrt
caryophyllene oxide	-0.0398	5.79E-01	No	0.0447	6.38E-01	No	NA

(Table C.5 continues)

Table C.5 (continued)

<b>Compound</b>	<b>Cor. with cap1</b>	<b>P cap1</b>	<b>Sig. cap1</b>	<b>Cor. with cap2</b>	<b>P cap2</b>	<b>Sig. cap2</b>	<b>Trans.</b>
cinnamic alcohol	-0.0780	2.71E-01	No	0.0626	4.86E-01	No	NA
Z-3-hexen-1-ol	0.1571	2.38E-02	No	-0.2750	3.52E-05	Yes	log
Z-3-hexenyl acetate	0.3767	1.18E-09	Yes	-0.3495	6.44E-08	Yes	log
Z- $\beta$ -ocimene	0.5282	1.51E-19	Yes	0.4436	5.16E-13	Yes	sqrt
Z-jasmone	0.0532	4.61E-01	No	-0.1381	7.85E-02	No	NA
Z-dimethylnonatriene	-0.0414	5.72E-01	No	9.23E-05	9.99E-01	No	NA
$\gamma$ -cadinene	0.2280	5.30E-04	Yes	0.0792	4.04E-01	No	sqrt
$\gamma$ -terpinene	0.1917	4.42E-03	Yes	-0.2311	6.27E-04	Yes	log
geraniol	-0.0717	3.09E-01	No	0.0739	4.52E-01	No	NA
germacrene D	0.0887	2.12E-01	No	-0.0691	4.81E-01	No	NA
intermedeol	-0.6455	1.73E-31	Yes	-0.0148	9.24E-01	No	sqrt
limonene	0.0222	7.44E-01	No	-0.1235	1.20E-01	No	NA
linalool	0.0772	2.71E-01	No	-0.1249	1.20E-01	No	NA
methyl nicotinate	0.2953	3.27E-06	Yes	0.1546	3.95E-02	No	log
methyl salicylate	0.0427	5.69E-01	No	0.1030	1.97E-01	No	NA
myroxide	0.1263	7.33E-02	No	0.2285	6.83E-04	Yes	log
para cymene	0.1540	2.65E-02	No	-0.1089	1.67E-01	No	NA
patchoulane	-0.0880	2.12E-01	No	-0.0426	6.38E-01	No	NA
sabinene	0.1382	4.80E-02	No	-0.1823	1.10E-02	No	NA
sabinene hydrate	0.1089	1.21E-01	No	-0.2749	3.52E-05	Yes	log
terpinen-4-ol	0.1183	9.16E-02	No	-0.1689	2.07E-02	No	NA
<i>E</i> -2-hexen-1-ol	-0.0839	2.34E-01	No	-0.0486	6.22E-01	No	NA
<i>E</i> - $\beta$ -caryophyllene	-0.0259	7.11E-01	No	-0.0531	5.77E-01	No	NA
<i>E</i> - $\beta$ -ocimene	0.5261	1.70E-19	Yes	0.4573	1.26E-13	Yes	sqrt

(Table C.5 continues)

Table C.5 (continued)

<b>Compound</b>	<b>Cor. with cap1</b>	<b>P cap1</b>	<b>Sig. cap1</b>	<b>Cor. with cap2</b>	<b>P cap2</b>	<b>Sig. cap2</b>	<b>Trans.</b>
<i>E</i> -cinnamic aldehyde	-0.2952	3.27E-06	Yes	0.0416	6.38E-01	No	sqrt
<i>E</i> -dimethylnonatriene	0.1181	9.16E-02	No	0.0675	4.85E-01	No	NA
<i>E-E-α</i> -farnesene	0.1436	4.11E-02	No	0.0029	9.99E-01	No	NA
unknown C <sub>15</sub> H <sub>24</sub> #1	0.1316	6.14E-02	No	-0.0058	9.79E-01	No	NA
unknown C <sub>15</sub> H <sub>24</sub> #2	-0.3326	1.05E-07	Yes	0.0706	4.78E-01	No	sqrt
unknown C <sub>15</sub> H <sub>24</sub> #3	-0.5157	1.04E-18	Yes	0.0122	9.27E-01	No	sqrt
veratrole	-0.2723	2.02E-05	Yes	0.0636	4.86E-01	No	sqrt
verbenone	-0.0122	8.58E-01	No	-0.1175	1.37E-01	No	NA

Table C.6. The values for the centroids for each species and community type along CAP axes 1 and 2.

<b>Species &amp; Community Type</b>	<b>CAP axis 1</b>	<b>CAP axis 2</b>
<i>C. cylindrica</i>		
Single-species communities	-1.006	0.137
Two-species communities	-0.678	0.196
Four-species communities	-0.547	-0.391
<i>C. unguiculata</i>		
Single-species communities	0.676	-1.212
Two-species communities	0.857	-0.061
Four-species communities	0.698	1.330

Table C.7. Tukey's Honest Significant Difference post-hoc tests on the differences between *C. cylindrica* and *C. unguiculata* in the given compound or compound class at each community type. The type of data transformation (log or square root) is indicated in the trait column. Compounds and compound classes are abbreviated as follows: SES: sesquiterpenoids; GLV: "green leafy volatiles;" AROM: aromatics; 2-APE: 2-amino phenyl ethanone;  $\alpha$ -P:  $\alpha$ -pinene;  $\beta$ -P:  $\beta$ -pinene;  $\gamma$ -T:  $\gamma$ -terpinene; SH: sabinene hydrate; MN: methyl nicotinate; (*E*)-C A: (*E*)-cinnamic aldehyde; V: veratrole; (*Z*)-3-H A: (*Z*)-3-hexenyl acetate. For log-transformed traits, tests are performed on the log odds scale such that ratios greater than one indicate that *C. cylindrica* has a higher emission rate than *C. unguiculata*, and ratios lower than one indicate that *C. unguiculata* has a higher emission rate. For square root-transformed traits, estimates are back-transformed from the square-root scale such that positive estimates indicate that *C. cylindrica* has a higher emission rate than *C. unguiculata*. General linear hypothesis tests determine the differences between the differences at a pair of community types. Positive estimates indicate the first community type in the hypothesis has a larger difference in the trait relative to the second community type in the hypothesis. All tests were corrected for multiple comparisons.

Tukey's Honest Significant Difference tests <i>Testing differences between species at com. types</i>					General linear hypothesis tests <i>Testing differences of the differences</i>			
Trait	Com. Type	Estimate or ratio $\pm$ 1 SE	<i>t</i> ratio	<i>P</i>	Hypo.	Estimate $\pm$ 1 SE	<i>Z</i> value	<i>P</i>
log SES	One	57.430 $\pm$ 21.44	10.85	<0.001	$\Delta$ One =	2.1868 $\pm$ 0.5229	4.18	< 0.001
	Two	6.450 $\pm$ 2.36	5.090	<0.001	$\Delta$ One =	1.0727 $\pm$ 0.5229	2.05	0.1001
	Four	19.650 $\pm$ 7.19	8.132	<0.001	$\Delta$ Four =	-1.1141 $\pm$ 0.5179	-2.15	0.0797
log GLV	One	0.070 $\pm$ 0.043	-4.269	0.001	$\Delta$ One =	1.043 $\pm$ 0.802	1.30	0.3935
	Two	0.025 $\pm$ 0.012	-7.370	< 0.001	$\Delta$ One =	-3.029 $\pm$ 0.802	-3.78	<0.001
	Four	1.439 $\pm$ 0.724	0.723	0.4704	$\Delta$ Two =	-4.073 $\pm$ 0.712	-5.72	< 0.001
sqrt AROM	One	-0.004 $\pm$ 0.011	-0.374	0.713	$\Delta$ One =	-0.041 $\pm$ 0.015	2.760	0.016
	Two	0.036 $\pm$ 0.010	3.662	<0.001	$\Delta$ Two =	-0.005 $\pm$ 0.015	-0.35	0.934
	Four	0.001 $\pm$ 0.010	0.115	0.909	$\Delta$ Four =	0.035 $\pm$ 0.014	-2.51	0.032
sqrt 2-APE	One	-0.009 $\pm$ 0.005	-1.855	0.0954	$\Delta$ One =	0.017 $\pm$ 0.006	2.918	0.009
	Two	-0.026 $\pm$ 0.003	-9.870	< 0.001	$\Delta$ Two =	0.021 $\pm$ 0.006	3.731	<0.001

(Table C.7 continues)

Table C.7 (continued)

Trait	Com. Type	Estimate or ratio $\pm$ 1 SE	<i>t</i> ratio	<i>P</i>	H	Estimate $\pm$ 1 SE	<i>Z</i> value	<i>P</i>
sqrt 2-APE	Four	-0.031 $\pm$ 0.003	-11.63	< <b>0.001</b>	$\Delta$ Two = $\Delta$ Four	0.005 $\pm$ 0.004	1.243	0.421
log $\alpha$ -P	One	0.213 $\pm$ 0.061	-5.406	< <b>0.001</b>	$\Delta$ One = $\Delta$ Two	-0.347 $\pm$ 0.341	-1.02	0.562
	Two	0.301 $\pm$ 0.056	-6.514	< <b>0.001</b>	$\Delta$ One = $\Delta$ Four	-1.002 $\pm$ 0.341	-2.94	<b>0.009</b>
	Four	0.580 $\pm$ 0.107	-2.958	<b>0.0034</b>	$\Delta$ Two = $\Delta$ Four	-0.656 $\pm$ 0.261	-2.51	<b>0.031</b>
log $\beta$ -P	One	0.627 $\pm$ 0.051	-5.765	< <b>0.001</b>	$\Delta$ One = $\Delta$ Two	-0.105 $\pm$ 0.107	-0.97	0.595
	Two	0.696 $\pm$ 0.049	-5.150	< <b>0.001</b>	$\Delta$ One = $\Delta$ Four	-0.358 $\pm$ 0.107	-3.34	<b>0.002</b>
	Four	0.897 $\pm$ 0.063	-1.538	0.125	$\Delta$ Two = $\Delta$ Four	-0.254 $\pm$ 0.099	-2.55	<b>0.029</b>
log $\gamma$ -T	One	0.579 $\pm$ 0.072	-4.390	< <b>0.001</b>	$\Delta$ One = $\Delta$ Two	0.044 $\pm$ 0.176	0.248	0.967
	Two	0.554 $\pm$ 0.069	-4.742	< <b>0.001</b>	$\Delta$ One = $\Delta$ Four	-0.495 $\pm$ 0.176	-2.81	<b>0.014</b>
	Four	0.949 $\pm$ 0.118	-0.420	0.675	$\Delta$ Two = $\Delta$ Four	-0.538 $\pm$ 0.176	-3.06	<b>0.006</b>
log SH	One	0.759 $\pm$ 0.055	-3.821	<b>0.002</b>	$\Delta$ One = $\Delta$ Two	-0.029 $\pm$ 0.096	-0.31	0.950
	Two	0.782 $\pm$ 0.050	-3.880	< <b>0.001</b>	$\Delta$ One = $\Delta$ Four	-0.276 $\pm$ 0.096	-2.87	<b>0.011</b>
	Four	1.000 $\pm$ 0.064	0	1	$\Delta$ Two = $\Delta$ Four	-0.246 $\pm$ 0.090	-2.74	<b>0.017</b>
log MN	One	0.783 $\pm$ 0.146	-1.313	0.216	$\Delta$ One = $\Delta$ Two	0.684 $\pm$ 0.228	2.999	<b>0.008</b>
	Two	0.395 $\pm$ 0.051	-7.086	< <b>0.001</b>	$\Delta$ One = $\Delta$ Four	0.494 $\pm$ 0.228	2.168	0.076
	Four	0.477 $\pm$ 0.063	-5.639	< <b>0.001</b>	$\Delta$ Two = $\Delta$ Four	-0.190 $\pm$ 0.185	-1.02	0.5603
sqrt ( <i>E</i> )-C A	One	0.008 $\pm$ 0.003	2.525	0.021	$\Delta$ One = $\Delta$ Two	-0.013 $\pm$ 0.004	-2.95	<b>0.009</b>
	Two	0.021 $\pm$ 0.003	6.859	< <b>0.001</b>	$\Delta$ One = $\Delta$ Four	-0.009 $\pm$ 0.004	-2.04	0.1033
	Four	0.016 $\pm$ 0.003	5.540	< <b>0.001</b>	$\Delta$ Two = $\Delta$ Four	0.004 $\pm$ 0.004	0.932	0.6199

(Table C.7 continues)

Table C.7 (continued)

Trait	Com. Type	Estimate or ratio $\pm$ 1 SE	<i>t</i> ratio	<i>P</i>	H	Estimate $\pm$ 1 SE	Z value	<i>P</i>
sqrt V	One	0.007 $\pm$ 0.003	2.175	0.042	$\Delta$ One =	-0.041 $\pm$ 0.004	-9.26	< <b>0.001</b>
	Two	0.047 $\pm$ 0.003	15.28	< <b>0.001</b>	$\Delta$ Two =	-0.023 $\pm$ 0.004	-5.22	< <b>0.001</b>
	Four	0.030 $\pm$ 0.003	9.567	< <b>0.001</b>	$\Delta$ Four =	0.018 $\pm$ 0.004	4.036	< <b>0.001</b>
log (Z)-3-H A	One	0.015 $\pm$ 0.009	-7.054	< <b>0.001</b>	$\Delta$ One =	-0.198 $\pm$ 0.697	-0.28	0.956
	Two	0.019 $\pm$ 0.007	-10.80	< <b>0.001</b>	$\Delta$ Two =	-4.520 $\pm$ 0.697	-6.48	< <b>0.001</b>
	Four	1.411 $\pm$ 0.520	0.935	0.351	$\Delta$ Four =	-4.322 $\pm$ 0.521	-8.30	< <b>0.001</b>

Table C.8. Outputs of ANOVAs for the nine compounds that had significant community type x species interactions. The trait column indicates the type of data transformation applied to the compound. Compounds are abbreviated as follows: 2-APE: 2-amino phenyl ethanone;  $\alpha$ -P:  $\alpha$ -pinene;  $\beta$ -P:  $\beta$ -pinene;  $\gamma$ -T:  $\gamma$ -terpinene; SH: sabinene hydrate; MN: methyl nicotinate; (*E*)-C A: (*E*)-cinnamic aldehyde; V: veratrole; (*Z*)-3-H A: (*Z*)-3-hexenyl acetate. *P* values for the community type x species interactions are adjusted for performing 23 tests (e.g. univariate analyses on all compounds that were significantly correlated with one or both of the first two CAP axes).

<b>Trait</b>	<b>Term</b>	<b>MS</b>	<b>NDF</b>	<b>DDF</b>	<b>F</b>	<b>P</b>
sqrt 2-APE	Type	0.001	2	7.287	3.450	0.088
	Species	0.017	1	21.403	110.540	6.51 E-10
	Type x Species	0.001	2	20.725	6.960	0.022
log $\alpha$ -P	Type	1.709	2	6.219	2.236	0.186
	Species	55.313	1	29.081	72.358	2.21 E-09
	Type x Species	4.175	2	22.979	5.461	0.038
log $\beta$ -P	Type	0.348	2	5.690	3.127	0.121
	Species	5.942	1	67.701	53.379	4.06 E-10
	Type x Species	0.693	2	40.299	6.225	0.022
log $\gamma$ -T	Type	1.270	2	264	3.640	0.028
	Species	10.614	1	264	30.410	8.33 E-08
	Type x Species	2.011	2	264	5.761	0.022
log SH	Type	0.385	2	7.247	4.245	0.060
	Species	1.862	1	83.568	20.537	1.93 E-05
	Type x Species	0.487	2	51.073	5.374	0.029
log MN	Type	1.262	2	6.647	3.263	0.103
	Species	20.454	1	38.803	52.900	9.33E-09
	Type x Species	1.748	2	28.389	4.521	0.050
sqrt ( <i>E</i> )-C A	Type	0.001	2	8.737	5.371	0.030
	Species	0.015	1	128.528	73.419	2.82E-14
	Type x Species	0.001	2	78.526	4.543	0.039
sqrt V	Type	0.009	2	264	43.138	< 2.2E-16
	Species	0.053	1	264	243.318	< 2.2E-16
	Type x Species	0.009	2	264	43.138	5.06 E-15
log ( <i>Z</i> )-3-H A	Type	5.014	2	8.530	1.642	0.249
	Species	299.506	1	35.599	98.049	9.12 E-12
	Type x Species	125.399	2	29.843	41.052	3.12 E-08

## APPENDIX D

### CHAPTER 4 APPENDIX

#### Appendix D-1: References cited in Table 4.1

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Table D.1. Communities sampled in 2015. *N* indicates the number of focal plants of each species that survived to fruiting at each community.

<b>Com. name</b>	<b>Latitude</b>	<b>Longitude</b>	<b>Number of species</b>	<b>Species present</b>	<b><i>N</i></b>
LCG	35.58201	-118.52308	1	<i>C. cylindrica</i>	31
SFL	35.58086	-118.52579	1	<i>C. cylindrica</i>	42
GUL	35.58163	-118.52162	1	<i>C. cylindrica</i>	35
S41	35.59493	-118.52420	1	<i>C. speciosa</i>	46
S60	35.58930	-118.50453	1	<i>C. speciosa</i>	32
TTI	35.59364	-118.50489	1	<i>C. speciosa</i>	23
BRO	35.59102	-118.51789	1	<i>C. xantiana</i>	39
CHG	35.53789	-118.64891	1	<i>C. xantiana</i>	45
ECR	35.58986	-118.44528	1	<i>C. xantiana</i>	47
PIT	35.52269	-118.66225	2	<i>C. cylindrica</i>	32
				<i>C. unguiculata</i>	39
RRO	35.52747	-118.65637	2	<i>C. cylindrica</i>	47
				<i>C. unguiculata</i>	24
BLG	35.59117	-118.52815	2	<i>C. speciosa</i>	20
				<i>C. xantiana</i>	35
GRC	35.59700	-118.50735	2	<i>C. speciosa</i>	33
				<i>C. xantiana</i>	41
GRE	35.59714	-118.50563	2	<i>C. speciosa</i>	29
				<i>C. xantiana</i>	41
S30	35.52542	-118.66633	3	<i>C. cylindrica</i>	37
				<i>C. speciosa</i>	16
				<i>C. unguiculata</i>	25
CPS	35.52172	-118.66689	3	<i>C. cylindrica</i>	38
				<i>C. speciosa</i>	41
				<i>C. unguiculata</i>	15
DEM	35.52891	-118.62655	4	<i>C. cylindrica</i>	33
				<i>C. speciosa</i>	27
				<i>C. unguiculata</i>	28
				<i>C. xantiana</i>	34
MCK	35.53633	-118.61418	4	<i>C. cylindrica</i>	30
				<i>C. speciosa</i>	37
				<i>C. unguiculata</i>	28
				<i>C. xantiana</i>	42

Table D.2. Communities sampled in 2017. For each of the two focal species (*C. cylindrica* and *C. xantiana*), there were three or four replicates of each of the three types of communities included in the study: one-species communities, two-species communities, and four-species communities. Species that were present in the community but not measured for this study are listed in brackets. *N* indicates the number of plants from which we recovered two undamaged fruits (neither the supplement nor the control fruits were damaged by herbivory).

Com. name	Latitude	Longitude	Number of species	Species present	<i>N</i>
SF	35.5809	-118.5258	1	<i>C. cylindrica</i>	54
MHG	35.5834	-118.5302	1	<i>C. cylindrica</i>	52
GUL	35.5816	-118.5216	1	<i>C. cylindrica</i>	60
DELW	35.5422	-118.6202	1	<i>C. xantiana</i>	45
BR	35.5910	-118.5179	1	<i>C. xantiana</i>	65
CG	35.5379	-118.6489	1	<i>C. xantiana</i>	65
SC	35.5294	-118.6460	2	<i>C. cylindrica</i> [ <i>C. unguiculata</i> ]	65
DVT	35.4742	-118.7286	2	<i>C. cylindrica</i> [ <i>C. unguiculata</i> ]	56
NP	35.5323	-118.6472	2	<i>C. cylindrica</i> [ <i>C. unguiculata</i> ]	65
S8	35.5908	-118.5120	2	<i>C. xantiana</i> [ <i>C. speciosa</i> ]	62
BG	35.5912	-118.5282	2	<i>C. xantiana</i> [ <i>C. speciosa</i> ]	39
GRC	35.5970	-118.5074	2	<i>C. xantiana</i> [ <i>C. speciosa</i> ]	53
DEM	35.5289	-118.6266	4	<i>C. cylindrica</i> <i>C. xantiana</i> [ <i>C. unguiculata</i> ] [ <i>C. speciosa</i> ]	61 45
DEMJR	35.5306	-118.6237	4	[ <i>C. cylindrica</i> ] <i>C. xantiana</i> [ <i>C. unguiculata</i> ] [ <i>C. speciosa</i> ]	63
MCK	35.5363	-118.6142	4	<i>C. cylindrica</i> <i>C. xantiana</i> [ <i>C. unguiculata</i> ] [ <i>C. speciosa</i> ]	44 43
DEL4	35.5464	-118.6170	4	<i>C. cylindrica</i> <i>C. xantiana</i> [ <i>C. unguiculata</i> ] [ <i>C. speciosa</i> ]	57 51

Table D.3. Selection differentials for all species in all replicate communities in 2015 and 2017.

Year	Species	Com. Type	Trait	Community	Estimate	Std. Error	t value	P		
2015	Cyl	One	AS	Lower Coyote Gulch	0.3654	0.4219	0.866	0.3885		
		One	AS	Sandy Flat	0.2229	0.2921	0.763	0.4470		
		One	AS	Upper Coyote Gulch	0.2303	0.3312	0.695	0.4885		
		One	Avg Size	Lower Coyote Gulch	-0.0661	0.2808	-0.235	0.8144		
		One	Avg Size	Sandy Flat	0.0311	0.2824	0.11	0.9120		
		One	Avg Size	Upper Coyote Gulch	0.2581	0.3258	0.792	0.4300		
		One	Fl Num	Lower Coyote Gulch	1.1989	0.1140	10.516	2.0E-16		
		One	Fl Num	Sandy Flat	1.5139	0.1283	11.798	<2e-16		
		One	Fl Num	Upper Coyote Gulch	0.9227	0.3033	3.043	0.0030		
		Two	AS	Pitch	-0.1482	0.1562	-0.949	0.3458		
		Two	AS	Rough Road	0.2028	0.1664	1.219	0.2270		
		Two	Avg Size	Pitch	0.0059	0.1785	0.033	0.9736		
		Two	Avg Size	Rough Road	0.2205	0.1750	1.26	0.2110		
		Two	Fl Num	Pitch	0.7512	0.1014	7.407	1.6E-10		
		Two	Fl Num	Rough Road	0.96264	0.0730	13.182	2.0E-16		
		Three	AS	S30	0.28613	0.2193	1.305	0.1962		
		Three	AS	Cattle Pens	0.04583	0.2409	0.19	0.8497		
		Three	Avg Size	S30	0.2919	0.2099	1.391	0.1687		
		Three	Avg Size	Cattle Pens	0.6957	0.2287	3.043	0.0033		
		Three	Fl Num	S30	1.4919	0.1957	7.622	8.4E-11		
		Three	Fl Num	Cattle Pens	1.1928	0.1856	6.425	1.3E-08		
		Four	AS	Democrat	0.2455	0.2609	0.941	0.3505		
		Four	AS	Mill Creek	0.6398	0.3661	1.748	0.0857		
		Four	Avg Size	Democrat	0.1404	0.3116	0.451	0.6539		
		Four	Avg Size	Mill Creek	-0.3674	0.2867	-1.281	0.2050		
		Four	Fl Num	Democrat	1.0468	0.1601	6.537	1.6E-08		
		Four	Fl Num	Mill Creek	1.12909	0.1790	6.309	4.0E-08		
			Spe	One	AS	41	0.4105	0.2395	1.713	0.0898
				One	AS	TipTop	0.02951	0.4590	0.064	0.9489

(Table D.3 continues)

Table D.3 (continued)

Year	Species	Com. Type	Trait	Community	Estimate	Std. Error	t value	P		
2015	Spe	One	AS	60	0.2654	0.4025	0.659	0.5112		
		One	Avg Size	41	0.7489	0.3793	1.974	0.0512		
		One	Avg Size	TipTop		0.0973	0.5452	0.178	0.8587	
		One	Avg Size	60		0.5904	0.5515	1.071	0.2870	
		One	Fl Num	41		2.6851	0.1271	21.122	2.0E-16	
		One	Fl Num	TipTop		0.4593	0.7693	0.597	0.5520	
		One	Fl Num	60		2.1502	0.2568	8.373	4.9E-13	
		Two	AS	GRC		0.883	0.4575	1.93	0.0573	
		Two	AS	GRE		-0.03741	0.4973	-0.075	0.9402	
		Two	AS	Black Gulch		0.883	0.4575	1.93	0.0573	
		Two	Avg Size	GRC		0.7334	0.3392	2.162	0.0337	
		Two	Avg Size	GRE		0.1851	0.3174	0.583	0.5615	
		Two	Avg Size	Black Gulch		0.7334	0.3392	2.162	0.0337	
		Two	Fl Num	GRC		0.9903	0.1520	6.516	7.0E-09	
		Two	Fl Num	GRE		1.1094	0.1482	7.486	1.1E-10	
		Two	Fl Num	Black Gulch		0.9903	0.1520	6.516	7.1E-09	
		Three	AS	S30		0.6517	0.4120	1.582	0.1197	
		Three	AS	Cattle Pens		0.5186	0.2759	1.88	0.0656	
		Three	Avg Size	S30		0.34277	0.4473	0.766	0.4468	
		Three	Avg Size	Cattle Pens		0.39463	0.2077	1.9	0.0629	
		Three	Fl Num	S30		0.97306	0.1645	5.916	2.5E-07	
		Three	Fl Num	Cattle Pens		1.47525	0.1194	12.361	2.0E-16	
		Four	AS	Democrat		0.42102	0.4999	0.842	0.4030	
		Four	AS	Mill Creek		0.32668	0.4407	0.741	0.4614	
		Four	Avg Size	Democrat		1.0236	0.6635	1.543	0.1281	
		Four	Avg Size	Mill Creek		0.2429	0.4122	0.589	0.5579	
		Four	Fl Num	Democrat		1.1954	0.2461	4.857	8.9E-06	
		Four	Fl Num	Mill Creek		1.6268	0.8129	2.001	0.0499	
		Ung	Two	AS	Pitch		-0.0188	0.4023	-0.047	0.9629
			Two	AS	Rough Road		0.1276	0.2265	0.564	0.5752
			Two	Avg Size	Pitch		-0.1198	0.2892	-0.414	0.6803
			Two	Avg Size	Rough Road		0.1579	0.2801	0.564	0.575
	Two		Fl Num	Pitch		1.8989	0.1519	12.502	2.0E-16	
	Two		Fl Num	Rough Road		0.4647	0.2678	1.735	0.0879	

(Table D.3 continues)

Table D.3 (continued)

Year	Species	Com. Type	Trait	Community	Estimate	Std. Error	t value	P
2015	Ung	Three	AS	S30	0.3442	0.2719	1.266	0.2136
		Three	AS	Cattle Pens	-0.4451	0.6377	-0.698	0.4900
		Three	Avg Size	S30	0.1165	0.4022	0.289	0.7739
		Three	Avg Size	Cattle Pens	-0.3388	0.3747	-0.904	0.3720
		Three	Fl Num	S30	1.3713	0.3076	4.458	7.8E-05
		Three	Fl Num	Cattle Pens	0.25	0.226	1.106	0.2760
		Four	AS	Democrat	0.1402	0.2563	0.547	0.5868
		Four	AS	Mill Creek	-0.02359	0.2317	-0.102	0.9193
		Four	Avg Size	Democrat	-0.05151	0.2568	-0.201	0.8418
		Four	Avg Size	Mill Creek	0.09023	0.2183	0.413	0.6810
	Four	Fl Num	Democrat	0.639	0.2439	2.619	0.0115	
	Four	Fl Num	Mill Creek	0.8393	0.1247	6.732	1.3E-08	
	Xan	One	AS	Borel	0.06673	0.1580	0.422	0.6735
		One	AS	China Gardens	0.16806	0.1944	0.865	0.3888
		One	AS	Erkine Creek	0.13933	0.1339	1.041	0.3000
		One	Avg Size	Borel	-0.06799	0.1832	-0.371	0.7112
		One	Avg Size	China Gardens	-0.1732	0.1500	-1.155	0.2503
		One	Avg Size	Erkine Creek	0.14954	0.1975	0.757	0.4505
		One	Fl Num	Borel	0.7831	0.3196	2.45	0.0157
		One	Fl Num	China Gardens	1.5423	0.0787	19.598	<2e-16
		One	Fl Num	Erkine Creek	1.52141	0.0963	15.802	<2e-16
		Two	AS	GRC	0.3849	0.2507	1.535	0.1280
		Two	AS	GRE	-0.03766	0.2154	-0.175	0.8615
		Two	AS	Black Gulch	-0.01703	0.2169	-0.079	0.9376
		Two	Avg Size	GRC	0.554	0.3231	1.715	0.0892
		Two	Avg Size	GRE	0.02083	0.1999	0.104	0.9172
		Two	Avg Size	Black Gulch	0.07278	0.2756	0.264	0.7922
		Two	Fl Num	GRC	1.13445	0.0762	14.894	<2e-16
		Two	Fl Num	GRE	1.45399	0.1078	13.485	<2e-16
		Two	Fl Num	Black Gulch	1.42285	0.1587	8.967	8.5E-15
		Four	AS	Democrat	0.4321	0.4389	0.985	0.3281
		Four	AS	Mill Creek	1.0712	0.5150	2.08	0.0411

(Table D.3 continues)

Table D.3 (continued)

Year	Species	Com. Type	Trait	Community	Estimate	Std. Error	t value	P
2015	Xan	Four	Avg Size	Democrat	-0.7768	0.6191	-1.255	0.2136
		Four	Avg Size	Mill Creek	0.4732	0.5251	0.901	0.3710
		Four	Fl Num	Democrat	2.23195	0.0932	23.947	2.0E-16
		Four	Fl Num	Mill Creek	1.85294	0.1186	15.624	2.0E-16
2017	Xan	One	AS	Borel	0.0864	0.1503	0.574	0.5670
		One	AS	China Gardens	0.09449	0.1842	0.513	0.6090
		One	AS	Delonegha W	-0.06611	0.1672	-0.395	0.6930
		One	Avg Size	Borel	0.18384	0.1671	1.1	0.2740
		One	Avg Size	China Gardens	-0.17854	0.1852	-0.964	0.3370
		One	Avg Size	Delonegha W	-0.03151	0.1269	-0.248	0.8040
		One	Fl Num	Borel	1.14627	0.0919	12.477	2.0E-16
		One	Fl Num	China Gardens	0.9107	0.1046	8.71	3.7E-14
		One	Fl Num	Delonegha W	0.71086	0.1183	6.011	2.5E-08
		Two	AS	Black Gulch	-0.11571	0.1323	-0.875	0.3833
		Two	AS	GRC	0.01387	0.1737	0.08	0.9365
		Two	AS	Site 8	0.1245	0.1636	0.761	0.4480
		Two	Avg Size	Black Gulch	0.26718	0.1735	1.54	0.1258
		Two	Avg Size	GRC	0.23223	0.1784	1.302	0.1950
		Two	Avg Size	Site 8	0.4613	0.1764	2.615	0.0099
		Two	Fl Num	Black Gulch	0.82564	0.0890	9.279	3.0E-16
		Two	Fl Num	GRC	0.79961	0.0599	13.34	<2e-16
		Two	Fl Num	Site 8	0.89831	0.0417	21.57	<2e-16
		Four	AS	Delonegha E	0.08482	0.1879	0.451	0.6521
		Four	AS	Mill Creek	0.09969	0.1366	0.729	0.4666
		Four	AS	Democrat	0.17397	0.1332	1.306	0.1932
		Four	AS	Democrat JR	-0.3151	0.1800	-1.751	0.0815
		Four	Avg Size	Delonegha E	-0.04625	0.1535	-0.301	0.7635
		Four	Avg Size	Mill Creek	0.0442	0.1565	0.283	0.7778
Four	Avg Size	Democrat	-0.15607	0.1609	-0.97	0.3331		
Four	Avg Size	Democrat JR	-0.1083	0.1761	-0.615	0.5393		
Four	Fl Num	Delonegha E	0.49811	0.0706	7.058	2.6E-11		
Four	Fl Num	Mill Creek	0.79328	0.0714	11.114	2.0E-16		

(Table D.3 continues)

Table D.3 (continued)

Year	Species	Com. Type	Trait	Community	Estimate	Std. Error	t value	P
2017	Xan	Four	Fl Num	Democrat	1.11192	0.0784	14.18	2.0E-16
		Four	Fl Num	Democrat JR	1.19563	0.0624	19.149	2.0E-16
	Cyl	One	AS	Upper Coyote Gulch	0.1717	0.1792	0.958	0.3400
		One	AS	Main Highway Gulley	-0.08571	0.1716	-0.5	0.6180
		One	AS	Sandy Flat	0.01372	0.2210	0.062	0.9510
		One	Avg Size	Upper Coyote Gulch	0.1995	0.1672	1.194	0.2344
		One	Avg Size	Main Highway Gulley	0.55427	0.2228	2.488	0.0139
		One	Avg Size	Sandy Flat	0.80906	0.2012	4.021	9.1E-05
		One	Fl Num	Upper Coyote Gulch	0.86021	0.0664	12.96	2.0E-16
		One	Fl Num	Main Highway Gulley	1.15486	0.0663	17.413	2.0E-16
		One	Fl Num	Sandy Flat	0.89739	0.0384	23.359	2.0E-16
		Two	AS	Divot	0.178	0.1256	1.417	0.1582
		Two	AS	North Pole	-0.09061	0.1441	-0.629	0.5304
		Two	AS	Summer Camp	0.08937	0.1236	0.723	0.4706
		Two	Avg Size	Divot	0.15771	0.1265	1.247	0.2140
		Two	Avg Size	North Pole	0.4325	0.1657	2.611	0.0098
		Two	Avg Size	Summer Camp	0.06748	0.1306	0.517	0.6060
		Two	Fl Num	Divot	1.18373	0.2911	4.066	7.1E-05
		Two	Fl Num	North Pole	0.89396	0.0687	13.006	2.0E-16
		Two	Fl Num	Summer Camp	0.93885	0.0674	13.932	2.0E-16
		Four	AS	Delonegha E	-0.1252	0.1297	-0.966	0.3358
		Four	AS	Mill Creek	-0.02078	0.1449	-0.143	0.8861
		Four	AS	Democrat	0.1333	0.1290	1.033	0.3032
		Four	Avg Size	Delonegha E	0.1166	0.1347	0.866	0.3880
		Four	Avg Size	Mill Creek	-0.04476	0.1985	-0.226	0.8218
		Four	Avg Size	Democrat	0.3499	0.1481	2.362	0.0194

(Table D.3 continues)

Table D.3 (continued)

Year	Species	Com. Type	Trait	Community	Estimate	Std. Error	t value	P
		Four	Fl Num	Delonegha E	0.66446	0.0783	8.483	1.6E-14
		Four	Fl Num	Mill Creek	0.83827	0.1947	4.306	2.9E-05
		Four	Fl Num	Democrat	1.12512	0.0732	15.37	2.0E-16

Table D.4. Descriptive statistics of anther-stigma separation (A.S), floral diameter (Avg.Size), and seed fitness (Seeds) for all species and replicate communities in 2015 and 2017.

Year	Trait	Com. Type	Community	Species	min	median	mean	max
2015	A.S	One	Lower Coyote Gulch	Cyl	0.9	2.56	2.5940	4.785
	A.S	One	Sandy Flat	Cyl	0.8	2.88	2.8406	5.51
	A.S	One	Upper Coyote Gulch	Cyl	1.2	3.07	3.0677	5.72
	Avg.Size	One	Lower Coyote Gulch	Cyl	7.995	16.005	16.416	28.49
	Avg.Size	One	Sandy Flat	Cyl	11.765	18.048	17.831	25.545
	Avg.Size	One	Upper Coyote Gulch	Cyl	13.275	19.355	19.391	24.075
	Fl.Num	One	Lower Coyote Gulch	Cyl	2	5	7.3226	20
	Fl.Num	One	Sandy Flat	Cyl	2	3	4.9048	20
	Fl.Num	One	Upper Coyote Gulch	Cyl	2	3	3.6286	9
	Seeds	One	Lower Coyote Gulch	Cyl	6	31.5	34.129	67
	Seeds	One	Sandy Flat	Cyl	0	33.25	34.595	96
	Seeds	One	Upper Coyote Gulch	Cyl	0	35	32.929	72
	A.S	Two	Pitch	Cyl	-2.52	1.73	1.7009	5.79
	A.S	Two	Rough Road	Cyl	-1.01	2.8	2.6932	6.58
	Avg.Size	Two	Pitch	Cyl	10.255	17.588	17.936	31.005
	Avg.Size	Two	Rough Road	Cyl	13.708	19.085	19.131	29.755
	Fl.Num	Two	Pitch	Cyl	2	3	5.1875	24
	Fl.Num	Two	Rough Road	Cyl	2	4	5.5957	26
	Seeds	Two	Pitch	Cyl	2	27.75	29.907	73
	Seeds	Two	Rough Road	Cyl	0	29	29.915	64.5
	A.S	Three	30	Cyl	-0.63	3.01	2.9042	6.95
	A.S	Three	Cattle Pens	Cyl	0.81	2.2425	2.6396	6.43
	Avg.Size	Three	30	Cyl	8.56	20.618	20.417	30.48
	Avg.Size	Three	Cattle Pens	Cyl	10.955	19.52	19.369	28.72
	Fl.Num	Three	30	Cyl	2	4	5.3158	20
	Fl.Num	Three	Cattle Pens	Cyl	2	5	5.4737	20
	Seeds	Three	30	Cyl	0	39	37.355	81
	Seeds	Three	Cattle Pens	Cyl	0	32	30.276	93

(Table D.4 continues)

Table D.4 (continued)

Year	Trait	Com. Type	Community	Species	min	median	mean	max
2015	A.S	Four	Democrat	Cyl	0.81	3.13	2.9995	5.25
	A.S	Four	Mill Creek	Cyl	1.17	2.8875	2.985	4.74
	Avg.Size	Four	Democrat	Cyl	11.005	17.76	17.984	24.265
	Avg.Size	Four	Mill Creek	Cyl	13.395	19.883	20.003	30.973
	Fl.Num	Four	Democrat	Cyl	2	4	4.9697	17
	Fl.Num	Four	Mill Creek	Cyl	1	4	4.8333	18
	Seeds	Four	Democrat	Cyl	4	36.5	33.727	67
	Seeds	Four	Mill Creek	Cyl	0	36.5	35.967	71.5
	A.S	One	41	Spe	1.63	4.165	4.7411	9.73
	A.S	One	60	Spe	1.44	4.065	4.06	7.9
	A.S	One	TipTop	Spe	0.98	3.81	3.9004	6.63
	Avg.Size	One	41	Spe	12.26	20.983	20.848	31.5
	Avg.Size	One	60	Spe	12.55	17.408	17.714	27.125
	Avg.Size	One	TipTop	Spe	12.4	16.71	17.367	24.805
	Fl.Num	One	41	Spe	1	3	3.8043	19
	Fl.Num	One	60	Spe	1	2	2.7188	9
	Fl.Num	One	TipTop	Spe	1	1	1.4348	4
	Seeds	One	41	Spe	0	39.5	37.682	82.141
	Seeds	One	60	Spe	0	25.642	30.063	87
	Seeds	One	TipTop	Spe	0	20	20.043	88
	A.S	Two	Black Gulch	Spe	2.06	4.85	4.81	47
	A.S	Two	GRC	Spe	1	2.98	3.2248	7.14
	A.S	Two	GRE	Spe	1.53	3.57	3.4248	4.96
	Avg.Size	Two	Black Gulch	Spe	13.205	22.7	22.893	28.79
	Avg.Size	Two	GRC	Spe	11.3	17.615	18.012	28.87
	Avg.Size	Two	GRE	Spe	11.825	16.475	17.414	27.34
	Fl.Num	Two	Black Gulch	Spe	1	4	6	17
	Fl.Num	Two	GRC	Spe	1	1	2.1212	17
	Fl.Num	Two	GRE	Spe	1	1	2.3793	24
	Seeds	Two	Black Gulch	Spe	6	37.75	37.775	71.5
	Seeds	Two	GRC	Spe	5	32	30.258	76.5
	Seeds	Two	GRE	Spe	1	22.5	25.603	55.5
	A.S	Three	30	Spe	1.55	3.57	3.7944	6.72
	A.S	Three	Cattle Pens	Spe	1.6	3.39	3.6095	7.72

(Table D.4 continues)

Table D.4 (continued)

Year	Trait	Com. Type	Community	Species	min	median	mean	max
2015	Avg.Size	Three	30	Spe	10.745	18.695	19.261	31.19
	Avg.Size	Three	Cattle Pens	Spe	9.5	17.98	18.329	27.46
	Fl.Num	Three	30	Spe	1	2	3.25	20
	Fl.Num	Three	Cattle Pens	Spe	1	3	4.3171	19
	Seeds	Three	30	Spe	4	25.25	26.625	50.5
	Seeds	Three	Cattle Pens	Spe	0	24	27.195	67
	A.S	Four	Democrat	Spe	0.89	2.86	3.1478	7.29
	A.S	Four	Mill Creek	Spe	1.02	3.18	3.4003	7.4
	Avg.Size	Four	Democrat	Spe	14.17	18.715	18.896	24.795
	Avg.Size	Four	Mill Creek	Spe	8.49	17.6	17.348	28.2
	Fl.Num	Four	Democrat	Spe	1	3	5.4444	28
	Fl.Num	Four	Mill Creek	Spe	1	2	2.0270	10
	Seeds	Four	Democrat	Spe	0	20	25.963	66.5
	Seeds	Four	Mill Creek	Spe	0	26	25.738	60
	A.S	Two	Pitch	Ung	1.42	3.11	3.3190	5.55
	A.S	Two	Rough Road	Ung	-2.74	2.12	2.4694	9.39
	Avg.Size	Two	Pitch	Ung	17.382 5	22.73	23.212	32.485
	Avg.Size	Two	Rough Road	Ung	15.445	23.442 5	24.367	32.525
	Fl.Num	Two	Pitch	Ung	1	7	8.9231	30
	Fl.Num	Two	Rough Road	Ung	4	8.5	9.7917	22
	Seeds	Two	Pitch	Ung	2	33	34.934	69.5
	Seeds	Two	Rough Road	Ung	3	19.25	20.188	45
	A.S	Three	30	Ung	-0.45	3.215	3.1688	6.09
	A.S	Three	Cattle Pens	Ung	0	2.2	2.0997	2.85
	Avg.Size	Three	30	Ung	17.02	22.895	22.745	26.705
	Avg.Size	Three	Cattle Pens	Ung	15.005	21.67	21.829	27.982 5
	Fl.Num	Three	30	Ung	3	6	8.24	24
	Fl.Num	Three	Cattle Pens	Ung	2	9	12.267	38
	Seeds	Three	30	Ung	1	24	25.34	48
	Seeds	Three	Cattle Pens	Ung	0	16	18.6	55
	A.S	Four	Democrat	Ung	0.88	2.8	3.0614	7.54
	A.S	Four	Mill Creek	Ung	-1.24	2.72	2.8895	7.12

(Table D.4 continues)

Table D.4 (continued)

Year	Trait	Com. Type	Community	Species	min	median	mean	max
2015	Avg.Size	Four	Democrat	Ung	14.915	21.7	21.704	31.675
	Avg.Size	Four	Mill Creek	Ung	8.835	25.34	24.854	31.685
	Fl.Num	Four	Democrat	Ung	2	8.5	10.214	26
	Fl.Num	Four	Mill Creek	Ung	3	12	15.714	55
	Seeds	Four	Democrat	Ung	4	22.75	23.362	49
	Seeds	Four	Mill Creek	Ung	0	28.5	27.834	54.5
	A.S	One	Borel	Xan	1.33	3.72	4.1792	7.83
	A.S	One	China Gardens	Xan	1.58	3.44	3.5504	6.32
	A.S	One	Erskine Creek	Xan	-1.88	3.84	3.8043	6.83
	Avg.Size	One	Borel	Xan	16.468	24.04	23.037	29.565
	Avg.Size	One	China Gardens	Xan	14.305	24.035	23.769	33.03
	Avg.Size	One	Erskine Creek	Xan	20.175	28.125	27.7	33.28
	Fl.Num	One	Borel	Xan	4	9	9.2821	15
	Fl.Num	One	China Gardens	Xan	4	12	17.089	57
	Fl.Num	One	Erskine Creek	Xan	4	15	17.894	53
	Seeds	One	Borel	Xan	3	32.611 6	30.766	57.5
	Seeds	One	China Gardens	Xan	0	34	34.033	71
	Seeds	One	Erskine Creek	Xan	0	46	48.735	85
	A.S	Two	Black Gulch	Xan	0.81	3.58	3.4843	7.22
	A.S	Two	GRC	Xan	1.99	3.725	3.9072	6.87
	A.S	Two	GRE	Xan	1.26	3.47	3.5909	6.6
	Avg.Size	Two	Black Gulch	Xan	16.02	23.215	22.882	31.265
	Avg.Size	Two	GRC	Xan	17.07	21.01	21.240	25.77
	Avg.Size	Two	GRE	Xan	14.59	22.665	22.204	29.365
	Fl.Num	Two	Black Gulch	Xan	4	10	14.086	49
	Fl.Num	Two	GRC	Xan	4	10	17.829	85
	Fl.Num	Two	GRE	Xan	3	9	13.439	75
	Seeds	Two	Black Gulch	Xan	2	30.5	29.314	57.5
	Seeds	Two	GRC	Xan	9.5	33	33.183	56.5
	Seeds	Two	GRE	Xan	0	25	25.268	53
	A.S	Four	Democrat	Xan	-0.67	4.1975	4.1612	7.61
	A.S	Four	Mill Creek	Xan	1.37	2.77	3.1895	6.89

(Table D.4 continues)

Table D.4 (continued)

Year	Trait	Com. Type	Community	Species	min	median	mean	max
2015	Avg.Size	Four	Democrat	Xan	16.845	26.661	26.149	34.82
	Avg.Size	Four	Mill Creek	Xan	14.37	24.71	24.151	30.233
	Fl.Num	Four	Democrat	Xan	2	14.5	23.735	163
	Fl.Num	Four	Mill Creek	Xan	2	13	18.881	125
	Seeds	Four	Democrat	Xan	0	39.5	39.838	83
	Seeds	Four	Mill Creek	Xan	0	43.455 26	38.974	73
2017	A.S	One	GUL	Cyl	1	2.79	2.7844	5.82
	A.S	One	MHG	Cyl	0.49	2.98	2.8348	5.42
	A.S	One	SF	Cyl	0.22	3.115	3.0458	5.99
	Avg.Size	One	GUL	Cyl	14.66	20.962 5	21.596	31.125
	Avg.Size	One	MHG	Cyl	12.87	21.845	21.955	29.025
	Avg.Size	One	SF	Cyl	11.19	22.495	22.235	29.605
	Fl.Num	One	GUL	Cyl	4	15	17.871	73
	Fl.Num	One	MHG	Cyl	3	17	19.291	57
	Fl.Num	One	SF	Cyl	2	16	22.298	153
	Seeds	One	GUL	Cyl	0	50.5	47.044	79
	Seeds	One	MHG	Cyl	1	50	46.965	84
	Seeds	One	SF	Cyl	0	46	42.776	81
	A.S	Two	DVT	Cyl	0.29	2.83	3.0535	6.71
	A.S	Two	NP	Cyl	0.09	2.91	2.9746	6.13
	A.S	Two	SC	Cyl	-1.87	2.345	2.2323	4.91
	Avg.Size	Two	DVT	Cyl	16.625	27.74	28.122	38.04
	Avg.Size	Two	NP	Cyl	14.2	19.505	20.110	31.28
	Avg.Size	Two	SC	Cyl	13	21.495	22.335	39.555
	Fl.Num	Two	DVT	Cyl	2	4	6	17
	Fl.Num	Two	NP	Cyl	3	17	20.813	77
	Fl.Num	Two	SC	Cyl	3	12	16.433	108
	Seeds	Two	DVT	Cyl	0	32.5	34.25	86
	Seeds	Two	NP	Cyl	0	56	54.415	97
	Seeds	Two	SC	Cyl	0	48.5	46.076	85
	A.S	Four	DEL4	Cyl	0.5	2.78	3.1371	7.57
	A.S	Four	DEM	Cyl	-1.71	1.985	1.8853	6.33
	A.S	Four	MCK	Cyl	-1.14	2.99	3.0394	5.85

(Table D.4 continues)

Table D.4 (continued)

Year	Trait	Com. Type	Community	Species	min	median	mean	max
2017	Avg.Size	Four	DEL4	Cyl	9.91	24.41	24.771	39.39
	Avg.Size	Four	DEM	Cyl	14.115	22.875	22.883	34.575
	Avg.Size	Four	MCK	Cyl	14.245	23.123	23.257	32.245
	Fl.Num	Four	DEL4	Cyl	2	18	19.915	110
	Fl.Num	Four	DEM	Cyl	2	18	21.444	77
	Fl.Num	Four	MCK	Cyl	6	17	17.091	36
	Seeds	Four	DEL4	Cyl	0	53.5	52.259	97
	Seeds	Four	DEM	Cyl	0	57	54.016	110
	Seeds	Four	MCK	Cyl	6	37	39.273	118
	A.S	One	BOR	Xan	0.31	3.43	3.3859	6.55
	A.S	One	CG	Xan	1.48	3.74	3.7543	7.44
	A.S	One	DELW	Xan	1.6	3.795	3.8292	6.02
	Avg.Size	One	BOR	Xan	21.56	27.26	26.992	32.575
	Avg.Size	One	CG	Xan	17.39	25.953	25.762	34.55
	Avg.Size	One	DELW	Xan	21.12	29.783	29.355	33.855
	Fl.Num	One	BOR	Xan	6	22	29.830	132
	Fl.Num	One	CG	Xan	7	35	41.625	139
	Fl.Num	One	DELW	Xan	7	29	35.829	82
	Seeds	One	BOR	Xan	8	50	48.313	81
	Seeds	One	CG	Xan	0	34	33.690	64
	Seeds	One	DELW	Xan	0	40	39.070	70
	A.S	Two	BG	Xan	0.73	3.74	3.7881	7.36
	A.S	Two	GRC	Xan	1.2	3.46	3.5124	7.22
	A.S	Two	S8	Xan	1.41	3.91	3.9453	7.27
	Avg.Size	Two	BG	Xan	19.595	25.92	25.955	32.795
	Avg.Size	Two	GRC	Xan	17.935	23.95	23.854	29.975
	Avg.Size	Two	S8	Xan	19.885	26.01	25.467	29.63
	Fl.Num	Two	BG	Xan	4	35	42.184	124
	Fl.Num	Two	GRC	Xan	4	31.5	40.250	155
	Fl.Num	Two	S8	Xan	9	44	58.869	282
	Seeds	Two	BG	Xan	8	39	38.188	63
	Seeds	Two	GRC	Xan	0	39	39	71
	Seeds	Two	S8	Xan	10	36.5	36.258	65

(Table D.4 continues)

Table D.4 (continued)

Year	Trait	Com. Type	Community	Species	min	median	mean	max
2017	A.S	Four	DEL4	Xan	1.67	3.825	3.7935	7
	A.S	Four	DEM	Xan	0.01	3.715	3.8657	7.55
	A.S	Four	DEMJR	Xan	2.31	3.795	4.1626	7.04
	A.S	Four	MCK	Xan	0.29	3.58	3.853	8.59
	Avg.Size	Four	DEL4	Xan	19.985	27.04	27.013	32.385
	Avg.Size	Four	DEM	Xan	17.115	27.325	26.856	34.12
	Avg.Size	Four	DEMJR	Xan	19.65	27.145	27.130	35.28
	Avg.Size	Four	MCK	Xan	20.84	27.97	27.905	33.11
	Fl.Num	Four	DEL4	Xan	3	17.5	27.125	155
	Fl.Num	Four	DEM	Xan	4	47	53.245	182
	Fl.Num	Four	DEMJR	Xan	3	26.5	40.733	167
	Fl.Num	Four	MCK	Xan	5	28	35.620	229
	Seeds	Four	DEL4	Xan	0	39	36.867	59
	Seeds	Four	DEM	Xan	0	52	48.895	89
	Seeds	Four	DEMJR	Xan	7	52	50.140	88
	Seeds	Four	MCK	Xan	0	36	37.194	74

Table D.5. Pearsons correlation coefficients for correlations between the three traits measured in this study, for each species in each year. *P* values are given in parentheses, and significantly correlations are in bold text.

Year	Species	Traits	A-S	Flower size
2015	<i>C. xantiana</i>	Flower size	<b>0.2085 (0.0002)</b>	
		Flower number	<b>0.1853 (0.0008)</b>	<b>0.2673 (&lt; 0.0001)</b>
2015	<i>C. cylindrica</i>	Flower size	<b>0.2613 (&lt; 0.0001)</b>	
		Flower number	0.0762 (0.1697)	0.0459 (0.4086)
2015	<i>C. speciosa</i>	Flower size	<b>0.3203 (&lt; 0.0001)</b>	
		Flower number	<b>0.2651 (&lt; 0.0001)</b>	<b>0.3171 (&lt; 0.0001)</b>
2015	<i>C. unguiculata</i>	Flower size	0.0044 (0.9557)	
		Flower number	0.0895 (0.2616)	0.0362 (0.6503)
2017	<i>C. xantiana</i>	Flower size	<b>0.1160 (0.0059)</b>	
		Flower number	0.0513 (0.2247)	-0.0478 (0.2577)
2017	<i>C. cylindrica</i>	Flower size	<b>0.2392 (&lt; 0.0001)</b>	
		Flower number	0.0226 (0.6080)	0.0435 (0.3228)

Table D.6. *F* values for the effect of the interaction between a trait and community type (number of species present in a community) on phenotypic selection of four *Clarkia* species in two years. Degrees of freedom are given in parentheses in front of each *F* value. Power values are observed power statistics; superscript crosses (<sup>‡</sup>) indicate *F* tests with non-significant interactions and variable selection across community types (see Table D.7), which may have been power-limited.

Year	Species	Trait	<i>F</i>	<i>P</i>	Power
2015	<i>C. cylindrica</i>	Anther stigma	(3, 317.00) 0.721	0.540	0.179 <sup>‡</sup>
		Floral diameter	(3, 317.00) 1.536	0.205	0.404
		Flower number	(3, 310.42) 4.340	<b>0.005</b>	0.878
2015	<i>C. speciosa</i>	Anther stigma	(3, 278.39) 1.393	0.245	0.337 <sup>‡</sup>
		Floral diameter	(3, 255.59) 7.865	<b>&lt;0.001</b>	0.766
		Flower number	(3, 248.54) 4.675	<b>0.003</b>	0.875
2015	<i>C. unguiculata</i>	Anther stigma	(2, 152.02) 2.988	0.053	0.569 <sup>‡</sup>
		Floral diameter	(2, 152.06) 1.216	0.299	0.088
		Flower number	(2, 151.72) 4.179	<b>0.017</b>	0.723
2015	<i>C. xantiana</i>	Anther stigma	(2, 316.58) 0.114	0.893	0.058
		Floral diameter	(2, 289.78) 2.138	0.120	0.085
		Flower number	(2, 315.95) 7.172	<b>0.001</b>	0.930
2017	<i>C. cylindrica</i>	Anther stigma	(2, 501.48) 4.781	<b>0.009</b>	0.316
		Floral diameter	(2, 477.00) 2.461	0.086	0.294
		Flower number	(2, 477.00) 0.484	0.616	0.079
2017	<i>C. xantiana</i>	Anther stigma	(2, 304.52) 0.401	0.670	0.302
		Floral diameter	(2, 304.54) 2.647	0.072	0.500
		Flower number	(2, 305.16) 2.472	0.086	0.119

Table D.7. Net selection ( $S \pm 1$  SE) on three floral traits by community type in 2015. The values in parentheses below each  $S \pm 1$  SE are bootstrapped 95 % confidence intervals. The first column contains the traits, which are abbreviated as: AS (anther-stigma separation), FD (floral diameter), FN (flower number). The second column contains the number of species present in the community (1, 2, 3, or 4 species). Blank cells indicate community type x species combinations that were not measured. Thick borders around all of the community types for a species indicate a significant interaction between the trait and community type for that species, and thin double lines indicate differences between community types for a trait that had a non-significant interaction with limited power (see Table D.5). Significance levels are: \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$  and are only indicated when the bootstrapped 95 % confidence interval does not contain zero.

Trait	Sp	<i>C. cylindrica</i>	<i>C. speciosa</i>	<i>C. unguiculata</i>	<i>C. xantiana</i>
AS	1	0.302 ± 0.126* (0.047, 0.290)	0.286 ± 0.093** (0.073, 0.318)		0.113 ± 0.083 (-0.017, 0.135)
	2	0.169 ± 0.089 (-0.007, 0.207)	0.162 ± 0.121 (-0.027, 0.270)	0.315 ± 0.122* (0.046, 0.392)	0.087 ± 0.098 (-0.037, 0.122)
	3	0.214 ± 0.104* (0.023, 0.223)	0.414 ± 0.147** (0.074, 0.385)	0.050 ± 0.194 (-0.170, 0.209)	
	4	0.008 ± 0.161 (-0.189, 0.182)	0.058 ± 0.123 (-0.176, 0.199)	-0.089 ± 0.113 (-0.160, 0.483)	0.151 ± 0.094 (-0.054, 0.187)
FD	1	0.202 ± 0.105 (-0.025, 0.254)	0.763 ± 0.119*** (0.320, 0.681)		0.037 ± 0.090 (-0.072, 0.139)
	2	0.120 ± 0.100 (-0.067, 0.172)	0.321 ± 0.099** (0.068, 0.373)	0.060 ± 0.127 (-0.116, 0.170)	0.099 ± 0.109 (-0.035, 0.135)
	3	0.326 ± 0.105** (0.069, 0.336)	0.302 ± 0.113** (0.048, 0.334)	-0.080 ± 0.219 (-0.304, 0.226)	
	4	-0.046 ± 0.150 (-0.058, 0.339)	0.299 ± 0.125 (-0.016, 0.360)	-0.047 ± 0.111 (-0.108, 0.076)	0.133 ± 0.111 (-0.041, 0.184)
FN	1	0.644 ± 0.090*** (0.273, 0.464)	0.817 ± 0.125*** (0.391, 0.947)		0.841 ± 0.113*** (0.288, 0.533)
	2	0.212 ± 0.087* (0.051, 0.214)	0.265 ± 0.093** (0.038, 0.537)	0.637 ± 0.150*** (0.166, 0.556)	0.538 ± 0.084*** (0.202, 0.350)
	3	0.408 ± 0.121*** (0.026, 0.423)	0.446 ± 0.114*** (0.174, 0.442)	0.119 ± 0.169 (-0.143, 0.447)	
	4	0.279 ± 0.124 (-0.058, 0.339)	0.323 ± 0.093*** (0.069, 0.386)	0.145 ± 0.098 (-0.111, 0.195)	0.365 ± 0.059*** (0.136, 0.315)

Table D.8. Net selection (S) on three pollination-related traits and across two pollination treatments at three types of communities for two *Clarkia* species in 2017. The values in parentheses below each  $S \pm 1$  SE are bootstrapped 95 % confidence intervals. *F* values test the effect of pollination treatment on selection, such that a significant *F* value indicates that selection differs between open- and hand-pollinated flowers. Thick borders around all of the community types for a species indicate a significant interaction between the trait and community type for that species (see Table D.7). Degrees of freedom are given in parentheses below each *F* value. Significance levels for the gradients:  $^{\circ} P < 0.1$ ,  $* P < 0.05$ ,  $** P < 0.01$ ,  $*** P < 0.001$  and are only indicated when the bootstrapped 95 % confidence interval does not contain zero.

Species	Trait	Comm. Type	Open pollination $S \pm 1$ SE (CI)	Hand pollination $S \pm 1$ SE (CI)	<i>F</i>	<i>P</i>
<i>C. xantiana</i>	Anther-stigma	One sp.	0.012 ± 0.043 (-0.051, 0.135)	-0.048 ± 0.037 (-0.126, 0.135)	5.718 (1, 117.13)	<b>0.018</b>
		Two sp.	0.016 ± 0.032 (-0.037, 0.062)	0.014 ± 0.030 (-0.028, 0.061)	0.174 (1, 150.09)	0.677
		Four sp.	0.022 ± 0.025 (-0.036, 0.087)	0.022 ± 0.024 (-0.038, 0.083)	0.030 (1, 215.83)	0.863
	Floral diameter	One sp.	-0.041 ± 0.039 (-0.119, 0.048)	-0.047 ± 0.036 (-0.117, 0.031)	1.089 (1, 114.61)	0.299
		Two sp.	0.090 ± 0.037* (0.038, 0.134)	0.107 ± 0.037** (0.056, 0.159)	1.785 (1, 146.80)	0.184
		Four sp.	0.025 ± 0.026 (-0.034, 0.073)	0.000 ± 0.025 (-0.050, 0.061)	0.593 (1, 214.49)	0.442
	Flower number	One sp.	-0.007 ± 0.049 (-0.118, 0.093)	0.004 ± 0.048 (-0.104, 0.087)	0.002 (1, 113.24)	0.962
		Two sp.	0.045 ± 0.028 (0.001, 0.108)	0.061 ± 0.028* (0.017, 0.119)	0.650 (1, 141.81)	0.422
		Four sp.	0.016 ± 0.025 (-0.044, 0.072)	0.024 ± 0.024 (-0.034, 0.086)	0.410 (1, 209.06)	0.523
<i>C. cylindrica</i>	Anther-stigma	One sp.	0.081 ± 0.046 $^{\circ}$ (0.007, 0.147)	0.108 ± 0.039** (0.056, 0.168)	0.131 (1, 163.96)	0.718
		Two sp.	0.122 ± 0.035*** (0.042, 0.195)	0.112 ± 0.031*** (0.028, 0.184)	2.041 (1, 196.50)	0.155
		Four sp.	-0.026 ± 0.034 (-0.095, 0.049)	0.016 ± 0.033 (-0.054, 0.076)	1.957 (1, 170.94)	0.164
	Floral diameter	One sp.	0.184 ± 0.050*** (0.099, 0.263)	0.160 ± 0.045*** (0.074, 0.224)	0.064 (1, 161.84)	0.801
		Two sp.	0.137 ± 0.037*** (0.064, 0.212)	0.145 ± 0.033*** (0.078, 0.213)	3.251 (1, 189.66)	0.073
		Four sp.	0.076 ± 0.040 (-0.026, 0.180)	0.090 ± 0.034 (-0.017, 0.200)	0.754 (1, 170.69)	0.387

(Table D.8 continues)

Table D.8 (continued)

Species	Trait	Comm. Type	Open pollination $S \pm 1 \text{ SE (CI)}$	Hand pollination $S \pm 1 \text{ SE (CI)}$	<i>F</i>	<i>P</i>
		One sp.	$0.125 \pm 0.035^{***}$ (0.053, 0.250)	$0.107 \pm 0.031^{***}$ (0.045, 0.201)	0.065 (1, 154.78)	0.799
<i>C. cylindrica</i>	Flower number	Two sp.	$0.101 \pm 0.038$ (-0.010, 0.192)	$0.140 \pm 0.033^{***}$ (0.087, 0.227)	1.193 (1, 184.37)	0.276
		Four sp.	$0.137 \pm 0.040^{**}$ (0.050, 0.255)	$0.111 \pm 0.035^{**}$ (0.030, 0.216)	0.184 (1, 159.63)	0.669

Table D.9. Density of *Clarkia* flowers (open flowers/m<sup>2</sup>) in 2015 and 2017. Species indicates the focal species under observation. The numbers in parentheses after each community name indicate the number of species present in the community.

Year	Species	Com.	Conspecific	Heterospecific	Relative conspecific
2015	<i>C. cylindrica</i>	LCG (1)	1.19 ± 0.25	–	–
		SF (1)	3.17 ± 0.30	–	–
		UCG (1)	4.80 ± 0.51	–	–
		PIT (2)	6.35 ± 1.08	0.83 ± 0.22	0.84 ± 0.05
		RR (2)	9.18 ± 0.93	0.34 ± 0.14	0.96 ± 0.02
		S30 (3)	5.98 ± 0.92	2.16 ± 0.51	0.74 ± 0.05
		CP (3)	6.12 ± 1.03	0.40 ± 0.09	0.91 ± 0.02
		DEM (4)	6.48 ± 0.93	0.53 ± 0.20	0.93 ± 0.02
2015	<i>C. speciosa</i>	MCK (4)	6.88 ± 0.77	1.59 ± 0.37	0.80 ± 0.04
		S41 (1)	3.63 ± 0.42	–	–
		S60 (1)	5.70 ± 0.77	–	–
		TT (1)	2.92 ± 0.39	–	–
		BG (2)	3.58 ± 0.85	0.65 ± 0.21	0.85 ± 0.05
		GRC (2)	2.58 ± 0.31	1.90 ± 0.66	0.75 ± 0.07
		GRE (2)	2.83 ± 0.42	0.75 ± 0.23	0.80 ± 0.05
		S30 (3)	1.08 ± 0.28	0.11 ± 0.06	0.79 ± 0.10
		CP (3)	1.13 ± 0.19	0.55 ± 0.20	0.79 ± 0.06
		DEM (4)	0.90 ± 0.15	0.64 ± 0.23	0.68 ± 0.07
2015	<i>C. unguiculata</i>	MCK (4)	1.07 ± 0.22	0.11 ± 0.10	0.92 ± 0.04
		PIT (2)	3.14 ± 0.36	0.31 ± 0.11	0.92 ± 0.03
		RR (2)	7.39 ± 0.90	0.75 ± 0.31	0.90 ± 0.04
		S30 (3)	9.85 ± 1.16	2.68 ± 0.98	0.87 ± 0.04
		CP (3)	2.87 ± 0.74	0.73 ± 0.40	0.86 ± 0.07
		DEM (4)	4.63 ± 0.76	2.28 ± 0.61	0.72 ± 0.06
2015	<i>C. xantiana</i>	MCK (4)	5.92 ± 1.13	2.26 ± 0.77	0.84 ± 0.05
		BR (1)	3.29 ± 0.58	–	–
		CG (1)	2.23 ± 0.33	–	–
		ECK (1)	3.30 ± 0.51	–	–
		BG (2)	1.17 ± 0.25	0.14 ± 0.10	0.91 ± 0.05
		GRC (2)	6.19 ± 0.60	0.46 ± 0.10	0.88 ± 0.03
		GRE (2)	1.77 ± 0.32	0.23 ± 0.09	0.86 ± 0.05
		DEM (4)	1.45 ± 0.41	0.79 ± 0.33	0.70 ± 0.08
MCK (4)	0.86 ± 0.22	0.04 ± 0.02	0.85 ± 0.07		

(Table D.9 continues)

Table D.9 (continued)

Year	Species	Com.	Conspecific	Heterospecific	Relative conspecific
2017	<i>C. cylindrica</i>	MHG (1)	28.72 ± 3.30	–	–
		SF (1)	46.70 ± 6.46	–	–
		UCG (1)	53.38 ± 5.65	–	–
		DVT (2)	209.09 ± 19.73	56.00 ± 6.63	0.73 ± 0.03
		NP (2)	90.00 ± 10.65	43.81 ± 6.52	0.65 ± 0.04
		SC (2)	51.11 ± 4.36	52.70 ± 9.37	0.62 ± 0.04
		DEL4 (4)	51.54 ± 4.81	67.45 ± 10.35	0.53 ± 0.04
		DEM (4)	52.62 ± 6.57	35.17 ± 4.96	0.61 ± 0.04
		MCK (4)	31.26 ± 4.03	55.60 ± 8.37	0.45 ± 0.05
2017	<i>C. xantiana</i>	BOR (1)	28.12 ± 5.67	–	–
		CG (1)	59.09 ± 6.60	–	–
		DELW (1)	15.90 ± 2.63	–	–
		BG (2)	15.73 ± 3.46	7.78 ± 2.24	0.70 ± 0.06
		GRC (2)	36.44 ± 4.33	7.79 ± 1.31	0.81 ± 0.05
		S8 (2)	28.18 ± 4.76	2.34 ± 0.69	0.86 ± 0.05
		DEL4 (4)	40.60 ± 3.98	4.00 ± 1.27	0.88 ± 0.03
		DEMJR (4)	41.90 ± 4.74	3.51 ± 1.51	0.94 ± 0.05
		DEM (4)	53.76 ± 6.19	8.10 ± 2.38	0.88 ± 0.03
		MCK (4)	11.02 ± 1.49	1.63 ± 0.57	0.88 ± 0.07

Table D.10. *F* values for the effect of absolute floral density (conspecific or heterospecific) on phenotypic selection of three floral traits of four *Clarkia* species in two years. Degrees of freedom are given in parentheses below each *F* value. Significance levels: °  $P < 0.1$ , \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ .

<b>Year</b>	<b>Species &amp; density measure</b>	<b>Anther-stigma</b>	<b>Floral diameter</b>	<b>Flower number</b>
2015	<i>C. cylindrica</i> Conspecific density	0.143 (1, 320.12)	2.818° (1, 311.15)	1.349 (1, 319.10)
	Heterospecific density	1.090 (1, 207.09)	4.846* (1, 205.57)	0.042 (1, 204.77)
2015	<i>C. speciosa</i> Conspecific density	0.983 (1, 299.67)	0.000 (1, 300.00)	7.065** (1, 294.86)
	Heterospecific density	0.039 (1, 183.8)	2.051 (1, 184.00)	0.003 (1, 184.00)
2015	<i>C. unguiculata</i> Conspecific density	0.800 (1, 152.56)	4.369* (1, 153.36)	2.206 (1, 153.84)
	Heterospecific density	3.802° (1, 150.81)	0.727 (1, 149.76)	9.477** (1, 148.94)
2015	<i>C. xantiana</i> Conspecific density	4.257* (1, 313.91)	3.732° (1, 296.14)	3.719° (1, 314.96)
	Heterospecific density	0.464 (1, 157.42)	1.967 (1, 155.04)	0.806 (1, 155.23)
2017	<i>C. cylindrica</i> Conspecific density	1.977 (1, 498.52)	1.117 (1, 502.79)	13.487*** (1, 502.33)
	Heterospecific density	2.816° (1, 340.35)	4.373** (1, 338.40)	2.232 (1, 342.59)
2017	<i>C. xantiana</i> Conspecific density	1.138 (1, 467.01)	0.533 (1, 470.95)	0.827 (1, 468.68)
	Heterospecific density	1.056 (1, 328.26)	0.023 (1, 329.38)	0.063 (1, 330.80)

Table D.11. Simple slopes analysis for the traits where density had a significant effect on patterns of selection for a given species in a given year (a significant density x trait interaction). Simple slopes were generally calculated at mean + SD (high), mean, and mean – SD values (exact numbers given in parentheses) except when these values fell outside of the observed density values. When the low density was negative, zero (0) was used as a low value. Estimates are the slope  $\pm$  1 SE for the relationship between the trait and fitness at the indicated density value. Significance levels:  $^{\circ}$ :  $P < 0.1$ , \*:  $P < 0.05$ , \*\*:  $P < 0.01$ .

Species	Year	Trait	Density measure	Density level (value)	Estimate $\pm$ 1 SE
<i>C. cylindrica</i>	2015	Floral diameter	Absolute heterospecific density	High (11.14)	0.00 $\pm$ 0.11
				Mean (3.79)	0.17 $\pm$ 0.06**
				Low <sup>+</sup> (0.00)	0.26 $\pm$ 0.07**
<i>C. speciosa</i>	2015	Flower number	Absolute conspecific density	High (21.4)	0.64 $\pm$ 0.10**
				Mean (10.29)	0.47 $\pm$ 0.06**
				Low <sup>+</sup> (0.00)	0.31 $\pm$ 0.06**
<i>C. unguiculata</i>	2015	Floral diameter	Absolute conspecific density	High (42.1)	-0.16 $\pm$ 0.12
				Mean (22.37)	0.01 $\pm$ 0.08
				Low (2.64)	0.18 $\pm$ 0.10 $^{\circ}$
<i>C. xantiana</i>	2015	Anther-stigma separation	Absolute conspecific density	High (22.99)	0.26 $\pm$ 0.09**
				Mean (10.34)	0.14 $\pm$ 0.05*
				Low <sup>+</sup> (0.00)	0.04 $\pm$ 0.06
<i>C. cylindrica</i>	2017	Flower number	Absolute conspecific density	High (153.59)	0.35 $\pm$ 0.06**
				Mean (69.34)	0.19 $\pm$ 0.03**
				Low <sup>+</sup> (0.00)	0.06 $\pm$ 0.03*
<i>C. cylindrica</i>	2017	Floral diameter	Absolute heterospecific density	High (111.66)	0.07 $\pm$ 0.04 $^{\circ}$
				Mean (51.14)	0.12 $\pm$ 0.03**
				Low <sup>+</sup> (0.00)	0.16 $\pm$ 0.04**

Table D.12. Trait and fitness means for open- and hand-pollinated flowers of *C. cylindrica* and *C. xantiana* studied in 2017. The mean difference between the open- and hand-pollination flowers was calculated from paired t-tests; positive values indicate that the open-pollinated flowers were larger than the hand-pollinated flowers. Significance levels: °  $P < 0.1$ , \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ .

Species	Com. Type/Com. (N)	Trait	Open-pollination	Hand-pollination	Mean difference
<i>C. cylindrica</i>	1/MHG (54)	Anther-stigma	2.826 ± 0.171	2.576 ± 0.154	-0.250
		Floral diameter	21.581 ± 0.394	21.952 ± 0.441	0.372
		Seeds/fruit	46.167 ± 2.583	49.833 ± 2.633	3.667
		Flower number	19.333 ± 1.699		
<i>C. cylindrica</i>	1/UCG (56)	Anther-stigma	2.866 ± 0.158	2.579 ± 0.154	-0.287*
		Floral diameter	21.637 ± 2.228	21.412 ± 0.497	-0.224
		Seeds/fruit	48.107 ± 0.506	48.464 ± 2.395	0.357
		Flower number	18.143 ± 1.637		
<i>C. cylindrica</i>	1/SF (47)	Anther-stigma	3.065 ± 0.153	2.975 ± 0.186	-0.090
		Floral diameter	22.601 ± 0.502	21.781 ± 0.537	-0.820*
		Seeds/fruit	43.383 ± 2.757	43.702 ± 2.771	0.319
		Flower number	22.298 ± 3.376		
<i>C. cylindrica</i>	2/DVT (56)	Anther-stigma	2.948 ± 0.207	2.755 ± 0.193	-0.193
		Floral diameter	28.210 ± 0.651	28.805 ± 0.719	0.596
		Seeds/fruit	34.250 ± 3.380	44.500 ± 3.436	10.250***
		Flower number	6.054 ± 0.545		
<i>C. cylindrica</i>	2/NP (64)	Anther-stigma	2.980 ± 0.158	2.776 ± 0.146	-0.204
		Floral diameter	20.074 ± 0.435	20.398 ± 0.440	0.324
		Seeds/fruit	55.266 ± 3.017	59.516 ± 2.796	4.250
		Flower number	20.813 ± 2.017		
<i>C. cylindrica</i>	2/SC (66)	Anther-stigma	2.245 ± 0.179	1.865 ± 0.171	-0.380
		Floral diameter	22.062 ± 0.534	22.691 ± 0.485	0.630
		Seeds/fruit	46.076 ± 2.535	56.939 ± 2.835	10.864***
		Flower number	16.621 ± 1.995		
<i>C. cylindrica</i>	4/DEL4 (56)	Anther-stigma	3.106 ± 0.211	3.228 ± 0.201	0.123
		Floral diameter	25.141 ± 0.644	25.336 ± 0.596	0.195
		Seeds/fruit	51.286 ± 3.349	59.179 ± 3.413	7.893***
		Flower number	20.589 ± 2.127		
<i>C. cylindrica</i>	4/DEM (63)	Anther-stigma	1.870 ± 0.189	1.783 ± 0.149	-0.088
		Floral diameter	22.905 ± 0.520	23.859 ± 0.658	0.954
		Seeds/fruit	54.016 ± 3.543	57.698 ± 3.071	3.683
		Flower number	21.444 ± 2.021		

(Table D.12 continues)

Table D.12 (continued)

Species	Community Type/ Community (N)	Trait	Open-pollination	Hand-pollination	Mean difference
<i>C. cylindrica</i>	4/MCK (42)	Anther-stigma	2.993 ± 0.253	2.846 ± 0.203	-0.146
		Floral diameter	23.006 ± 0.585	23.022 ± 0.586	0.015
		Seeds/fruit	39.619 ± 3.296	45.452 ± 3.350	5.833*
		Flower number	17.548 ± 1.145		
<i>C. xantiana</i>	1/BOR (42)	Anther-stigma	3.105 ± 0.172	3.255 ± 0.199	0.149
		Floral diameter	27.297 ± 0.360	27.762 ± 0.454	0.465
		Seeds/fruit	48.262 ± 2.339	50.714 ± 2.421	2.452
		Flower number	29.619 ± 3.850		
<i>C. xantiana</i>	1/CG (33)	Anther-stigma	3.510 ± 0.179	3.414 ± 0.234	-0.096
		Floral diameter	25.886 ± 0.465	25.886 ± 0.465	0.140
		Seeds/fruit	35.697 ± 3.228	38.364 ± 3.172	2.667
		Flower number	39.848 ± 4.320		
<i>C. xantiana</i>	1/DELW (40)	Anther-stigma	3.772 ± 0.162	3.736 ± 0.199	-0.096
		Floral diameter	29.115 ± 0.498	29.226 ± 0.429	0.140
		Seeds/fruit	39.525 ± 2.564	37.725 ± 2.477	-2.667
		Flower number	34.925 ± 3.142		
<i>C. xantiana</i>	2/BG (41)	Anther-stigma	3.832 ± 0.249	3.869 ± 0.321	0.037
		Floral diameter	25.947 ± 0.430	26.321 ± 0.435	0.374
		Seeds/fruit	39.488 ± 1.835	41.195 ± 2.024	1.707
		Flower number	41.829 ± 3.778		
<i>C. xantiana</i>	2/GRC (43)	Anther-stigma	3.576 ± 0.181	3.621 ± 0.166	0.044
		Floral diameter	23.568 ± 0.399	24.217 ± 0.379	0.650***
		Seeds/fruit	39.628 ± 2.153	38.163 ± 1.940	-1.465
		Flower number	39.674 ± 5.344		
<i>C. xantiana</i>	2/S8 (61)	Anther-stigma	3.877 ± 0.135	3.873 ± 0.136	-0.004
		Floral diameter	25.563 ± 0.283	26.109 ± 0.262	0.546
		Seeds/fruit	36.262 ± 1.387	37.213 ± 1.242	0.951
		Flower number	58.869 ± 5.403		
<i>C. xantiana</i>	4/DEL4 (60)	Anther-stigma	3.743 ± 0.130	3.755 ± 0.175	0.013
		Floral diameter	26.963 ± 0.375	27.295 ± 0.337	0.332
		Seeds/fruit	36.867 ± 1.890	35.400 ± 1.945	-1.467
		Flower number	26.817 ± 4.115		
<i>C. xantiana</i>	4/DEM (42)	Anther-stigma	3.796 ± 0.262	3.852 ± 0.265	0.055
		Floral diameter	26.623 ± 0.513	26.739 ± 0.541	0.117
		Seeds/fruit	50.833 ± 2.673	49.452 ± 2.861	-1.381
		Flower number	52.690 ± 5.310		

(Table D.12 continues)

Table D.12 (continued)

<b>Species</b>	<b>Community Type/ Community (N)</b>	<b>Trait</b>	<b>Open-pollination</b>	<b>Hand-pollination</b>	<b>Mean difference</b>
<i>C. xantiana</i>	4/DEMJR (49)	Anther-stigma	4.228 ± 0.166	4.093 ± 0.187	-0.135
		Floral diameter	27.093 ± 0.401	26.823 ± 0.409	-0.269
		Seeds/fruit	50.592 ± 2.307	50.878 ± 2.278	0.286
		Flower number	41.510 ± 5.706		
<i>C. xantiana</i>	4/MCK (60)	Anther-stigma	3.758 ± 0.178	3.605 ± 0.199	-0.153
		Floral diameter	27.749 ± 0.368	27.722 ± 0.388	-0.027
		Seeds/fruit	37.517 ± 2.307	34.767 ± 2.046	-2.750
		Flower number	36.417 ± 4.069		

Table D.13. Back-transformed model estimated means and 95% confidence intervals for pollinator visitation data. These data were fit with a tweedie glmer using the lme4 and statmod packages in R. The power variance function was a Poisson and the power link function was a log-link. The dependent variable was the number of pollinator visits that occurred in a 15 minute observation window, and it was modeled as a function of community type (fixed effect) and plot nested within community (random effects). The means and confidence interval limits were back-transformed by exponentiating the means for each level of the community type factor, which were estimated using the emmeans function from the emmeans package in R. The contrasts column reflects the significance of pairwise comparisons between community type levels conducted in emmeans. Within a species, community type levels with different letters reflect contrasts that were significant at  $P < 0.05$  after adjusting for multiple comparisons, e.g. visitation to *C. cylindrica* was significantly lower at two-species communities than it was at one- or four-species communities.

<b>Species</b>	<b>Community Type</b>	<b>Mean</b>	<b>Lower CI limit</b>	<b>Upper CI limit</b>	<b>Contrasts</b>
<i>C. xantiana</i>	One	0.2069	0.1088	0.3937	a
	Two	0.3538	0.1945	0.6437	a
	Four	0.1782	0.1006	0.3155	a
<i>C. cylindrica</i>	One	0.1701	0.0811	0.3566	a
	Two	0.0115	0.0019	0.0707	b
	Four	0.1679	0.0802	0.3512	a